

How Absence Seizures Impair Sensory Perception: Insights from Awake fMRI and Simulation Studies in Rats

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Abstract

Absence seizures are characterized by regular and generalized spike-and-wave electrical patterns in the brain, resulting in unresponsiveness to environmental stimuli. In patients suffering absence epilepsy, recurring seizures can significantly decrease their quality of life and lead to yet untreatable comorbidities. The mechanism underlying the reduced responsiveness to external stimulus remains unknown.

This study aimed to investigate whole-brain responsiveness to visual and somatosensory whisker stimulation in GAERS, a well-established rat model for absence epilepsy. Animals were imaged continuously using a quiet zero-echo-time functional magnetic resonance imaging (fMRI) sequence while in a non-curarized awake state, allowing for naturally occurring seizures to be produced inside the 9.4T magnet. Sensory stimulations were applied in 28 fMRI sessions during interictal and ictal periods, as assessed by concurrent EEG recordings, and whole brain responsiveness and hemodynamic responses were compared between these two states. Additionally, a mean-field simulation model was used to mechanistically explain the changes of neural responsiveness to visual stimulation between interictal and ictal states.

Results showed that whole-brain responses to both sensory stimulations were suppressed and spatially hindered during a seizure. In several cortical regions, hemodynamic responses were negatively polarized during seizures, despite the application of a stimulus. The simulation results agreed well with fMRI findings, showing restricted propagation of spontaneous activity due to stimulation. These results suggest that typical information flow in functional pathways responsible for processing sensory stimulation is hindered and suppressed during absence seizures, potentially contributing to decreased responsiveness.

eLife assessment

This study used fMRI in an animal that simulates a type of epilepsy to ask if the responsiveness of the brain was altered during a seizure. The results suggest that the brain responsiveness is reduced during the seizures. The significance of the findings is **useful** and the strength of the evidence is **incomplete** but could be strengthened after additional experiments.

1. Introduction

Absence seizures typically appear in children between the ages of 5 and 7, and are characterized by a sudden, brief impairment of consciousness, an interruption from ongoing activities, and an unresponsiveness to environmental stimuli (Loiseau, Duché, and Pédespan 1995 [↗](#)). These seizures typically last for a few seconds to several minutes and include a regular electroencephalographic pattern known as spike-and-wave discharge (SWD) with a frequency of 2-5 Hz (Szaflarski et al. 2010 [↗](#)). In non-treated patients, absence seizures can occur from a few to hundreds of times per day (Loiseau, Duché, and Pédespan 1995 [↗](#)). The impaired consciousness during absence seizures can be highly disabling. Indeed, absence epilepsy is not a benign condition and is often accompanied by severe neuropsychiatric comorbidities including impairment of attention, memory and mood (Crunelli et al. 2020 [↗](#)). To better understand how these co-morbidities arise, it is important to investigate how information processing is altered between the ictal and interictal periods.

In absence epilepsy, human neuroimaging studies suggest that during a seizure, there may be a lack of conscious information processing due to impaired fronto-parietal network, arousal systems in the thalamus and brainstem (Blumenfeld 2012 [↗](#)), or default mode network (Luo et al. 2011 [↗](#)). Likewise, multiple human studies on absence seizure have demonstrated lack of responsiveness to external stimuli such as commands and questions, potentially caused by focal disruption of information processing in specific corticothalamic networks (Blumenfeld 2012 [↗](#)). However, to our knowledge, current neuroimaging studies on absence epilepsy have been conducted in a resting state, without external stimulus. Therefore, it remains unclear how the brain manages environmental stimuli during absence discharges, and more research is needed to understand how the brain responsiveness is affected during altered brain states.

The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is a well-established model of absence epilepsy. It is based on the selection of rats exhibiting spontaneous SWD and recapitulates most electrophysiological, behavioral and pharmacological features of human absence epilepsy. (Depaulis, David, and Charpier 2016 [↗](#)). Although the origin of absence seizures is not fully understood, current studies on rat models of absence seizures suggest that they arise from atypical excitatory-inhibitory patterns in the barrel field of the somatosensory cortex (Meeren et al. 2002 [↗](#); Polack et al. 2007 [↗](#)) and lead to synchronous cortico-thalamic activity (Holmes, Brown, and Tucker 2004 [↗](#)).

Previous results on GAERS have indicated that during an absence seizure, hyperactive electrophysiological activity in the somatosensory cortex can contribute to bilateral and regular SWD firing patterns throughout the cortex. These patterns propagate to different cortical areas (retrosplenial, visual, motor and secondary sensory), basal ganglia, cerebellum, substantia nigra and thalamus (David et al. 2008 [↗](#); Polack et al. 2007 [↗](#)). Graph based analyses have showed a network shift during an absence seizure, most notable by increased segregation of cortical regions from the rest of the brain (Wachsmuth et al. 2021 [↗](#)). Although seizures are initially triggered by

hyperactive somatosensory cortical neurons, the majority of neuronal populations are deactivated rather than activated during the seizure, resulting in an overall decrease in neuronal activity during SWD (McCafferty et al. 2023 [↗](#)). In addition, a previous fMRI study on GAERS, which measured changes in cerebral blood volume, found both deactivated and activated brain areas during seizures (David et al. 2008 [↗](#)). These alterations in neuronal activity patterns during seizures may also affect responses to concurrent stimuli and therefore could contribute to the alterations in behavioral responsiveness observed in animal models of epilepsy (Chipaux et al. 2013 [↗](#); Luo et al. 2011 [↗](#); Meeren et al. 2002 [↗](#); Studer et al. 2019 [↗](#))

The objective of this study was to investigate changes in whole brain responsiveness to sensory stimuli during ictal and interictal states, using the GAERS animal model. To avoid the potential impact of anesthetic agents on fMRI recordings and because absence seizures can be only observed in awake animals, EEG-fMRI was performed in GAERS trained to remain still and awake, *i.e.* non-curarized and non-anesthetized, using a previously validated protocol (Paasonen et al. 2018 [↗](#); P. Stenroos et al. 2018 [↗](#)). Visual and somatosensory whisker stimulations were used during both ictal and interictal states, as previous research has shown altered electrophysiological activity and behavior in these sensory systems during seizures (Meeren et al. 2002 [↗](#); Meyer et al. 2018 [↗](#); Pavone et al. 2001 [↗](#); Studer et al. 2019 [↗](#)). By using both stimulation schemes, we aimed to investigate alterations in brain responsiveness in each sensory system and to identify any common changes. To further describe the change when switching between ictal and interictal states, the whole-brain response to visual stimulus in each state was simulated, using a recent mean-field model (Volo et al. 2019 [↗](#)).

2. Methods

2.1. Animals

All experiments were approved by the local animal welfare committee (Comité Local GIN, C2EA-04) and complied with EU guidelines (Directive 2010/63/EU). Every precaution was taken to minimize the number of animals used and stress to animals during experiments. A total of 11 adult 8–12-month age old GAERS rats were used (260 ± 21 g), equally divided by gender. After implantation of an electroencephalography (EEG) lead (see below), rats were individually housed in their cages. Animals were maintained on a 12/12 h light-dark cycle at room temperature of $22 \pm 2^\circ\text{C}$, humidity of 50–60%. Food and water were available *ad libitum*.

2.2. EEG implantation

Carbon fiber (WPI Sarasota FL) electrodes were prepared as follows. Fiber leads were cut to small (~30 mm in length) parts and the end of the electrode was exposed from insulating cover to leave a brush-like, ~5 mm-length, tip. Rats were anesthetized with isoflurane (induction with 5%, a percentage gradually decreased to 1–2% for the surgery maintenance). The head was then shaved to remove the fur above the scalp, and the rat was positioned in a stereotaxic frame (David Kopf Instruments, Germany). After local lidocaine chlorhydrate injections (2g/100ml, 0,05 ml/site), the skull was exposed, cleaned with sterile 0.9% saline and hydrogen peroxide, and allowed to dry. Small holes were drilled halfway through the skull and carbon fiber electrodes were laid and glued with cyanoacrylate over the right motor cortex (AP: +2, ML: + 2,5 mm) and right primary somatosensory cortex (AP: -2,5, ML: + 3 mm) for seizure detection. An electrode working both as a reference and ground was placed on top of the cerebellum (AP: -12, ML: + 2 mm). The other end of electrode leads was inserted inside a plastic tube, which was positioned at the center of the skull. A thin layer of cyanoacrylate was applied to cover the skull. On top of the glue, a layer of dental cement (Selectaplust, DeguDent GmbH, Germany) was applied to finish the implant. Following the surgery, rats were individually caged to recover, and the welfare of the animals was closely monitored.

2.3. Animal habituation for awake imaging

A low noise zero echo time (ZTE) MRI sequence was used with awake rats aiming for low-stress and low-motion functional imaging sessions that allows rats to produce spontaneous seizures. For the habituation, peak ZTE scanner noise was measured with an omnidirectional condenser microphone (MT830R, Audio-Technica Limited, Leeds, UK) and Audacity software (version 2.3.0, <https://www.audacityteam.org/>), similar to (Paasonen et al. 2020) and reproduced at equivalent sound pressure through a loudspeaker.

To habituate the rats for the fMRI experiments, a procedure based on a previous study was followed (Stenroos et al. 2018). Restraint parts compatible with standard Bruker rat bed and suitable for stimulation leads were designed using 123D CAD software (Autodesk, San Rafael, CA) and 3D-printed with Ultimaker 2 (Utrecht, Netherlands) using Acrylonitrile Butadiene Styrene (ABS) plastic (Figure 1). The rats' bodies were restrained with a soft and elastic foamed plastic, hind legs were taped together, and front legs were taped loosely together to the side of the body. Front teeth were secured with a carbon fiber teeth bar, head with a nose cone, and neck and shoulders with a neck and shoulder bars, respectively. Silicone ear plugs were used to minimize experienced noise. Rats were habituated to restraint and MRI ZTE gradient noises by gradually increasing session times from 15 minutes to 60 minutes per day for 7-8 days before the first fMRI experiments. The length of habituation period was selected based on pilot experiments and was sufficient for rats to be in low-stress state and produce absence seizures inside the magnet. Respiration rate and motion were monitored during habituation sessions using a pressure pillow and video camera to estimate stress level. During the last habituation session, EEG was measured to confirm that the rats produced a sufficient amount of absence seizures (10 or more per session). Total of 2-5 fMRI experiments were conducted per rat within a 1–3-week period. In case rats were re-imaged more than 1 week after the preceding experiment, an additional 2-day habituation period was conducted. Before and after each habituation session, rats were given a treat of sugar water and/or chocolate cereals as positive reinforcement.

2.4. EEG-MRI protocol

MRI acquisitions were carried out at 9.4 T (Biospec Avance III HD, Bruker, Ettlingen, Germany; IRMaGe facility) using Paravision 7. In-house made transmit-receive loop coil with a 22 mm inner diameter was designed to host the EEG lead and to be compatible with the rat restraining cradle (Figure 2). 3D-printed sledge was designed to cover the circuit board of the coil and partly stabilize coaxial cable and the loop. Before connecting carbon fiber electrode leads, the loop was placed around the implant and sledge was fastened to the animal cradle with masking tape. Next, electrode leads were connected to the other end of the EEG cable. The cable was carefully secured on top of 3D-holder, without touching the animal to avoid breathing and motion artifacts.

2.4.1 fMRI optimization

To select the optimal fMRI sequence for the study design, standard gradient-echo echo-planar imaging (GE-EPI) and zero echo time imaging (ZTE; see section 2.4.3 for details) were compared. Peak acoustic noise levels produced by the MRI scanner during the sequence run, level of spatial distortions on MRI images caused by electrodes, gradient switching artifacts on EEG, and functional contrast were evaluated (Figure 2, Figure 2-figure supplement 1). Motion incidences between this study and previous study using EPI sequence (Stenroos et al. 2018) were compared. ZTE was selected over EPI sequence as it was quieter, produced less susceptibility artifacts, had less noise on EEG recordings and led to less animal motion (see Results section). Eventually, the ZTE parameters were adapted to optimize the functional contrast to noise ratio together with the temporal and spatial resolutions, based on previous reports (MacKinnon et al. ISMRM 2021) and our own pilot acquisitions. Note that ZTE is sensitive to change in blood flow (Lehto et al. 2017) and not to BOLD contrast as in EPI, and therefore also more direct measure of neuronal activation.

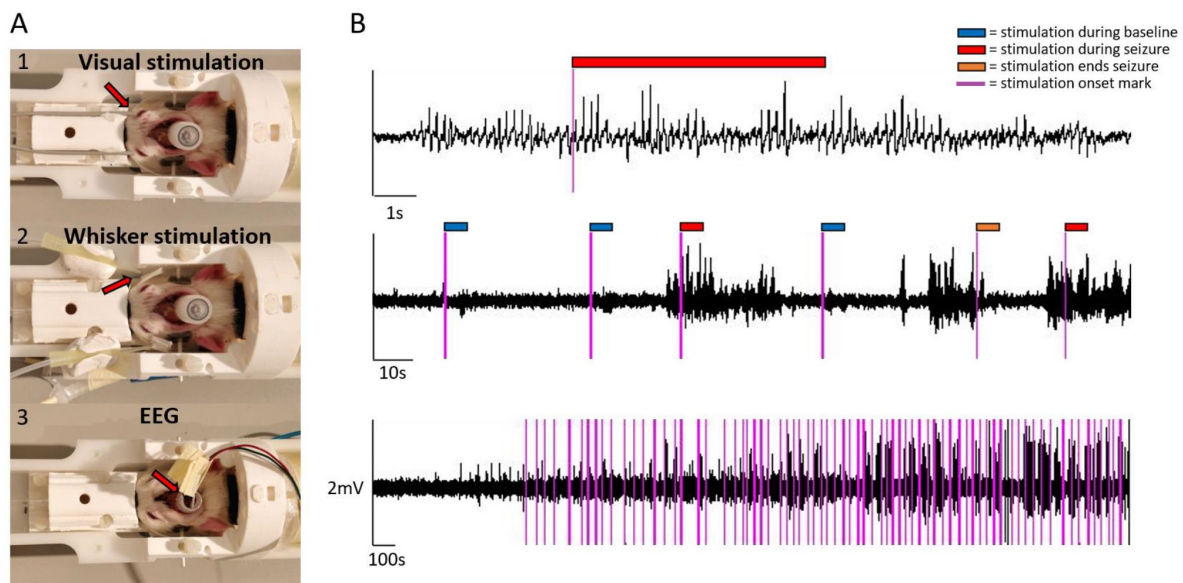


Figure 1.

Sensory stimulation, EEG setup and example EEG traces.

For visual stimulation, optical fiber cables were positioned bilaterally close to the eyes (A1); For whisker stimulation, plastic tips guided air flow bilaterally to whiskers (A2). For EEG, carbon fiber leads were connected to electrodes coming from a plastic tube (A3). EEG traces are illustrated at 3 different temporal scale, during ictal and interictal states and with stimulation onset marks (B). Color tags mark stimulation onsets (purple) and the 6-second stimulation blocks during baseline (blue), seizure (red) and when stimulation ended a seizure (orange). The onset marks were added post-hoc, based on recorded TTL events.

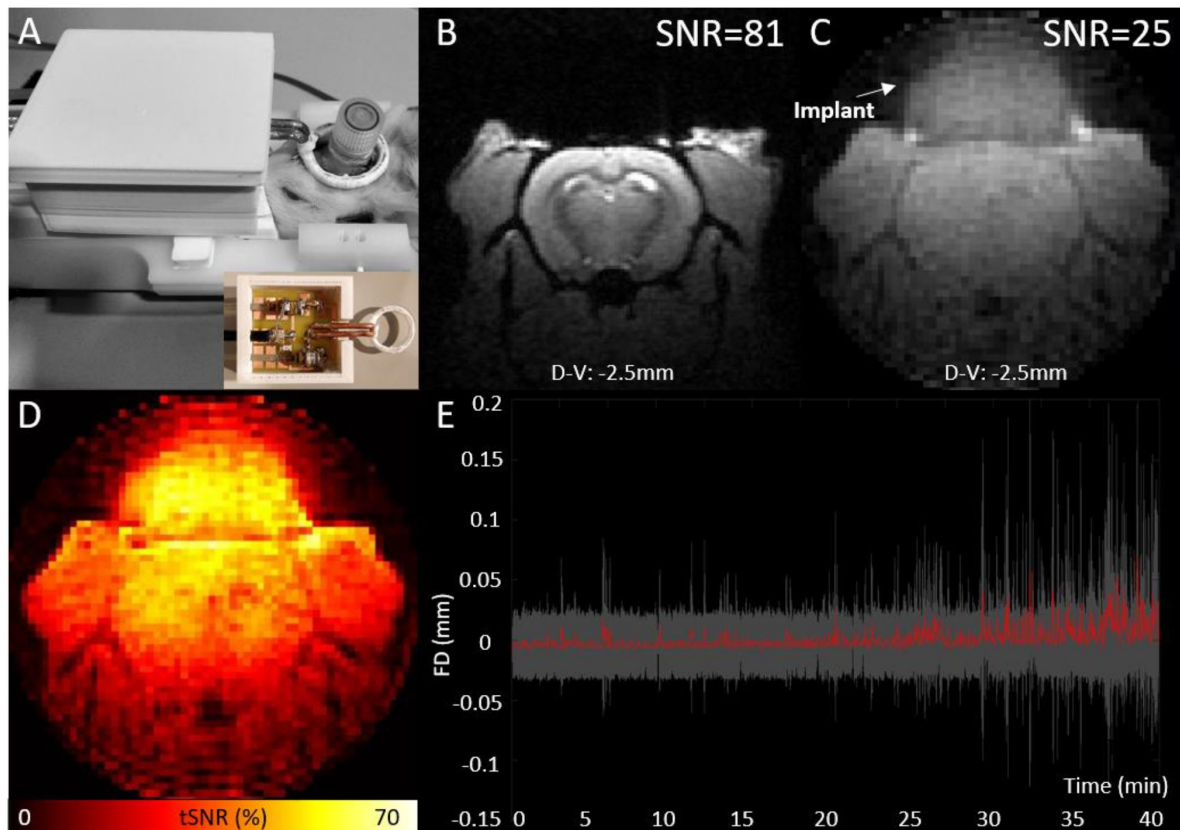


Figure 2.

EEG-fMRI setup and illustration of MRI-data quality.

MRI transmit-receiver loop coil placed around the implant (A), spatial signal-to-noise ratios of an illustrative anatomical (B), and ZTE image (C) and temporal signal-to-noise ratios (tSNR) of ZTE data (D) from a one example animal. Average framewise displacement (red) with the standard deviation (grey area) across all sessions included to analyses (E).

2.4.2 Animal installation

Before awake EEG-fMRI measurements, rats were anesthetized with isoflurane (5% induction, 2% maintenance in 30% O₂/ 70% N₂). Next, animals were wrapped and taped for awake imaging (section 2.3 [↗](#)) and the animals were moved to the MRI scanner and restrained to 3D-printed holder. The respiration rate was measured with a pressure pillow placed under animals and a Biopac amplifier system (Goleta, CA, USA). Temperature was not measured in awake condition to avoid causing any harms to the rectum due to motion. We relied on pilot calibration of the heated water to maintain the body temperature around 37 °C. Lastly, EEG leads were connected, and animals were pushed to the center of the magnet bore. After manual tuning and matching of the transmit-receive coil, shimming and anatomical imaging, isoflurane was turned off. Once animals woke up from anesthesia, they were left to rest in a quiet bore for 5-15 min so that spontaneous seizures started to emerge, after which functional imaging was started.

2.4.3. EEG-MRI acquisition and stimulations parameters

Anatomical imaging was conducted with a T1-FLASH sequence (TR: 530 ms, TE: 4 ms, flip angle 18°, bandwidth 39,682 kHz, matrix size 128 × 128, 51 slices, field-of-view 32 × 32 mm², resolution 0.25 × 0.25 × 0.5 mm³). fMRI was performed with a 3D ZTE sequence (TR: 0.971 ms, TE: 0 ms, flip angle 4°, pulse length 1 μs, bandwidth 150 kHz, oversampling 4, matrix size 60 × 60 × 60, field-of-view 30 × 30 × 60 mm³, resolution of 0.5 × 0.5 × 1 mm³, polar under sampling factor 5.64, nr. of projections 2060 resulting to a volume acquisition time of about 2 s). A total of 1350 volumes (45 min) were acquired.

Visual (n=14 sessions, 5 rats) and somatosensory whisker (n=14 sessions, 4 rats) stimulations fMRI measurements were performed (**Figure 1a** [↗](#)). Stimulus duration, frequency and pulse lengths were automatically controlled by an Arduino chip (Arduino Uno Rev3). For the visual stimulation, light pulses (3 Hz, 6 s total length, pulse length 166 ms) were produced by a blue led and light was guided through two optical fibers to the front of the rat's eyes.

For the somatosensory stimulation, an air-pressure valve (PMI-200 pressure micro-injector, Dagan corporation, Minneapolis, USA) controlled by the Arduino chip produced air flow pulses (2 Hz, 6 s total length, pulse length 250 ms) with a pressure of 2.3 bar. Air-flow was guided through polyethylene tubes, ending with a plastic tip, in front of the rat's whiskers, which were maintained together with a piece of tape. Stimulation parameters were based on previous rat stimulation fMRI studies to produce robust responses (Van Camp et al. 2006 [↗](#); Lu et al. 2016 [↗](#)). Both sets of stimulations were initiated manually using the live EEG recording as a guide, either during ictal or interictal state, to pursue equal sampling of both states across each 45-min fMRI session. A delay of at least 20 s was maintained between each stimulation block, to allow hemodynamic responses to settle to baseline between each block. EEG was recorded with a sampling rate of 1024 Hz (Micromed, SD MRI amplifier, Treviso, Italy). EEG recording software received a TTL trigger from the MRI scanner to mark the MRI sequence onset and TTL triggers from the stimulator to mark each stimulation period onset. During the live monitoring, a 50 Hz notch filter, and a low pass filter of 12 Hz facilitated online visual detection of seizures.

2.5. Data analysis

2.5.1 Motion analysis during fMRI

Motion occurrences were analyzed by visually inspecting image volumes through each scan and average motions per minute were calculated. Motion correction parameters given by ANTs (see 2.5.3.) were used and maximum value was taken to estimate maximum displacement of the head

from each session. Framewise displacement (FD) (**Figure 2E**) was calculated by taking the sum of absolute of differential of all motion parameters (Power et al. 2012), using 5 mm as estimate of the rat brain radius.

2.5.2 EEG analysis

EEG data was converted from native Micromed TRC-file format to mat- and dat-files using SPM toolbox ImaGIN (<https://github.com/manikbh/ImaGIN2>). Data were filtered with a 50 Hz notch and 1-90 Hz Butterworth band pass filters using SPM and ImaGIN. Absence seizures were manually inspected from a filtered signal and spectrogram. Seizures were confirmed as SWDs if they had a typical regular pattern, had at least double the amplitude compared to baseline signal and were within the 7-12 Hz frequency range. Two successive seizures were counted as one if there was less than 1 s of baseline signal between them. Seizure was counted as absence seizure only if it lasted at least 2 s, since behavioral deficits are not obvious in shorter seizures (Blumenfeld 2012). Seizure initiation and ending time points as well as stimulation onsets were marked in seconds and converted to MRI time in volumes.

Temporal registration between EEG and fMRI was performed using TTL triggers delivered by the MRI sequence and registered as the same time as EEG signals.

2.5.3 fMRI analysis

To prepare the fMRI data for analysis, data were motion corrected, co-registered with anatomical MRI, and spatially smoothed to reduce noise level. The preprocessing steps were performed using advanced normalizing tools (ANTs, <http://stnava.github.io/ANTs/>) and python based graphical user interphase mri_works (https://montigno.github.io/mri_works/Home/index.html). The first 5 volumes from each fMRI data were removed to allow signal to reach steady state. For motion correction, all volumes were transformed to an average image taken from first 10 volumes by using antsMotionCorr. Normalization was performed indirectly and using the T1-FLASH images, as ZTE images lack anatomical contrast. First, the T1-FLASH images of each animal were corrected from intensity non-uniformity using N4BiasFieldCorrection from ANTs. Next, brain was extracted with MP3 software (Brossard et al. 2020). T1-FLASH images of animals were then co-registered on top of reference T1-FLASH image (taken from a representative animal from our study) with ANTs using rigid, affine and SYN registrations. Transformation matrices were applied to ZTE images by ANTsApplyTransform so that ZTE images were eventually aligned with the reference T1-FLASH image. Finally, ZTE-images were smoothed with a Gaussian filter (1 mm full width at half maximum).

Post-hoc, stimulation periods were retrospectively classified into three different types, based on the relative position in time of the stimulation period with respect to the brain state (**Figure 1B**). Stimulation applied fully during an ictal period and those during an interictal period were used for the ictal-interictal comparison analysis. However, there were also intermediate cases where the seizure started or ended during the stimulation block (Figure 1–figure supplement 1). These intermediate cases were modeled as confounds in the SPM analysis of fMRI data (see 2.5.3.) and thus explained variance that was not accounted for by the main effects of interest. Additionally, a contrast was given to two cases: i) when stimulation ended a seizure (0-2 s between stimulation start and seizure end) and ii), when a seizure occurred, in absence of stimulation.

In a first level of SPM analysis, parameters were estimated in each brain state using the onset and duration of stimulations and seizures as inputs (Figure 1–figure supplement 1). Stimulations that did not fully coincide with a seizure were considered as nuisance regressors in the second level analysis. Motion periods were also included as multiple regressors (not convolved with a basis function) to be used as nuisance regressors. Stimulations that coincided with a motion above 0.3% of the voxel size were not considered as stimulation inputs. Stimulation and seizure inputs were convolved with “3 gamma distribution basis functions” (i.e. 3rd order gamma) in SPM (option:

basis functions, gamma functions, order: 3), to account for temporal and dispersion variations in the hemodynamic response. The choice of 3rd order gamma was based on the expectation that time-to-peak and shape of HRFs of seizure could vary across voxels (David et al. 2008 [↗](#)).

Three regressors were formed from each stimulation type and from seizures, and parameter estimates of beta-values were calculated for every voxel. HRFs due to stimulation and seizures were also calculated from selected ROIs (**Figure 4** [↗](#)) by multiplying each of the three gamma basis functions with its corresponding average beta value over a ROI and summing them up. The shape and time-to-peak of HRFs during an interictal condition were in line with previous literature (Lambers et al. 2020 [↗](#)), thereby confirming the selection of the 3rd order gamma basis function to model HRF. Extreme beta-values (maximum or minimum), calculated from a sum of each gamma derivative's beta-values in a selected ROI, between different conditions were compared using a two-tailed t-test (**Figure 4** [↗](#)).

In a second group analysis level, contrasts were added to parameter estimates (beta-values) as a function of the studied stimulation type. For example, to compare the effect of ictal-interictal stimulations, the previously derived beta values of each animal, corresponding to ictal stimulation, were given a contrast of 1, and that corresponding to interictal stimulation were given a contrast of - 1. Similarly, contrast to parameters estimates of beta-values corresponding to the seizure itself or stimulation that immediately ended seizure were studied. Statistical F-contrast maps were created and corrected for multiple comparisons by cluster level correction (**Figure 3** [↗](#) and **5** [↗](#)). F-contrast was used to test differences of any gamma function derivatives. For difference maps between ictal-interictal stimulations, F-contrast maps were masked with a T-contrast map, where only 1st order gamma derivative was used, to reveal brain areas where ictal stimulation provided higher amplitude response than interictal stimulation.

2.6 Modeling and Simulations

Whole brain simulations of the rat brain during sensory stimulation were performed. Each region of the rat brain was modelled via a recently developed mean-field model of Adaptive Exponential LIF neurons (AdEx) (Di Volo et al. 2019 [↗](#)), which describes the activity of a population of neurons made by excitatory and inhibitory cells (for details on the model see Sup. Information and Di Volo et al. 2019 [↗](#)). The AdEx mean-field model is capable of describing the neuronal dynamics during normal (interictal) and ictal periods (see results section). The interictal periods were modeled by an asynchronous irregular (AI) type of dynamics, while ictal periods were modeled by an oscillatory dynamic which resembles the spike-and-wave discharges (SWD) observed during absence epilepsy. The mean-field model was combined with the BAMS rat connectome (Bota, Dong, and Swanson 2012 [↗](#)) to build a realistic whole-brain simulation of the stimulus propagation during ictal and interictal periods. The simulations were made with the Virtual Brain platform (TVB), which provides a framework to perform large-scale brain simulations (Sanz-Leon et al. 2015 [↗](#)).

3. Results

3.1. Fine-Tuning of fMRI recording in awake rats

As GAERS rats only experience seizures when they are awake and in a quiet state, we used a low noise zero echo time (ZTE) MRI sequence. Spatial and temporal signal to noise ratios (SNRs) were assessed in one representative rat (**Figure 2** [↗](#)). Spatial SNR was obtained as the ratio between the mean signal intensity in an area of interest (the cortex) and the standard deviation of the background signal, whereas temporal SNR was obtained voxel-wise by dividing the mean by the standard deviation of the normalized fMRI signal. The spatial SNR was ~25 whereas the temporal

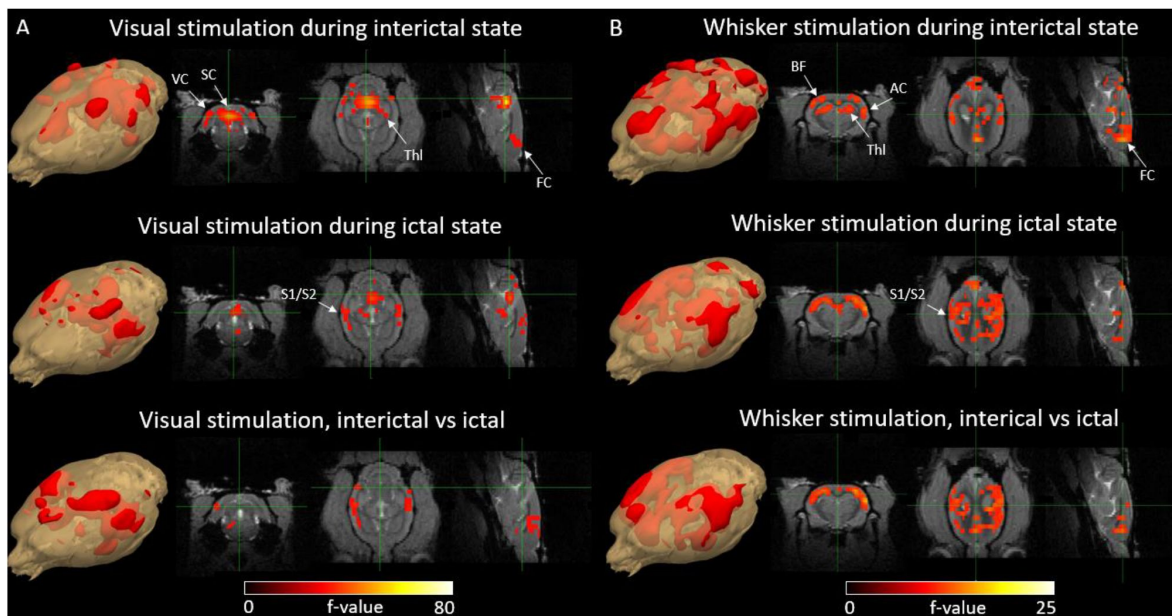


Figure 3.

Activation F-contrast maps of stimulation responses during interictal and ictal states and difference maps between these two states for the visual (A) and the whisker (B) stimulation experiments.

Parameter estimates of regressors were calculated for every voxel, and contrasts were added to parameter estimates of interictal stimulation, ictal stimulation and to compare interictal versus ictal stimulation in visual (A) and whisker (B) stimulation groups. For statistical significance, F-contrast ($p < 0.05$) maps were created and corrected for multiple comparisons by cluster level correction. AC = auditory cortex, BF = barrel field, FC = frontal cortex, SC = superior colliculus, S1/S2 = primary and secondary somatosensory cortex, Thl = thalamus, VC = visual cortex.

SNR was between 30-60 in the brain (**Figure 2**). In pilot studies, responses to visual stimulation measured using ZTE and EPI sequences were compared in one animal (Figure 2–figure supplement 1).

Average signal change due to stimulation was between 1-1.5% with gradient-echo-EPI sequence while it was ~0.3% with ZTE. However, activation maps (conducted with Aedes, <https://github.com/mjnissi/aedes>) demonstrated less susceptibility-induced image distortions in ZTE sequence compared to EPI. Moreover, when studying the effect of fMRI artifacts on EEG signal, more pronounced and higher amplitude gradient switching artifacts were detected when using EPI compared to ZTE sequence.

When comparing peak acoustic noise levels inside the 9.4T Bruker magnet, ZTE (78.7 dB) was 35.8 dB quieter than EPI (114.5 dB) equalling ~62 times weaker sound pressure (Schomer 1998). Motion during fMRI imaging using ZTE was low (**Figure 2b**). Mean framewise head translation was only $0.69 \pm 0.32 \mu\text{m}$ and head rotation $0.79 \pm 0.55^\circ$. Maximum displacement of the rat head was $12.8 \pm 11.6 \mu\text{m}$, corresponding to 0.03 ± 0.02 voxels, while maximum rotation was $18.1 \pm 12.9^\circ$, which demonstrates relatively low motion. Motion occurrences between the current study (0.43 ± 0.45 motions/min) and the previous study (1.0 ± 0.20 motions/min), performed with a similar rat restraint holder but an EPI sequence (P. Stenroos et al. 2018), demonstrated the advantage of using the quieter ZTE sequence compared to the louder EPI sequence. ZTE motion levels were similar to that obtained in a previous study using a quiet MB-SWIFT sequence (0.48 ± 0.23 motions/min, Paasonen et al. 2020).

In our study, use of 3 out of 11 animals (27%) had to be discontinued due noisy EEG signal, most likely caused by partly detached implant. From MRI-measurements, 6 out of 28 sessions (21%) needed to be excluded from analyses due to an excessive movement that prevented reliable fMRI analysis. Moreover, 4 sessions (14%) were excluded due to lack of seizures, and 1 session (4%) was excluded due to technical failure in providing air-puffs to whiskers. Respiration of awake animals remained stable during the measurements. Average respiration frequency across the 45-min scans was 2.2 ± 0.4 Hz in visual stimulation group and 2.0 ± 0.3 Hz in whisker stimulation group and no sudden changes of respiration due to stimulations were noted.

3.2. Response to sensory stimuli

Stimulations were manually initiated during ictal and interictal periods, but some stimulation blocks co-occurred unintentionally in-between ictal and interictal periods. **Table 1** shows the amount of each stimulation type and the amount and duration of seizure periods.

Statistical activation maps (**Figure 3**) in response to stimulus were created in interictal and ictal periods and compared between these two states. During interictal condition in the visual stimulation group, statistical responses ($p < 0.05$, cluster-level corrected) were most notably seen in the visual cortex, the superior colliculus, the thalamus (including the lateral geniculate nucleus) and the frontal cortex (including the prelimbic, cingulate and secondary motor cortices). However, during a seizure condition, responses in the visual cortex were less pronounced while responses in the superior colliculus remained stable. In addition, some responses were observed in the somatosensory cortex during a seizure state, probably due to incomplete nuisance removal of the effect of the seizure itself by the linear model used. There were more activated voxels during interictal state compared to ictal state (136% more). Comparing the statistical responses between interictal and ictal states revealed significant changes ($p < 0.05$, cluster-level corrected) in the visual, somatosensory, and frontal cortices, with these regions being less activated during the ictal than during the interictal state (see also **Figure 4**).

During the interictal condition in the whisker stimulation group, responses ($p < 0.05$, cluster-level corrected) were most notably seen in the somatosensory cortex, including the barrel field, the auditory cortex, the thalamus, including the ventral posteromedial nucleus, and the frontal cortex.

Visual stimulation group			Whisker stimulation group		
Stimulations (nr/45min)			Stimulations (nr/45min)		
	Mean	SD		Mean	SD
Stimulation during baseline	32.4	12.3	Stimulation during baseline	22.2	8.8
Stimulation fully inside a seizure	11.4	8.2	Stimulation fully inside a seizure	9.9	9.5
Stimulation started after a seizure, 50% inside a seizure	2.9	3.0	Stimulation ended after a seizure, 50% inside a seizure	2.1	2.2
Stimulation started after a seizure, 50% outside a seizure	3.9	2.5	Stimulation ended after a seizure, 50% outside a seizure	3.5	4.0
Stimulation started before seizure, 50% inside a seizure	1.5	1.6	Stimulation started before seizure, 50% inside a seizure	0.1	0.3
Stimulation started before seizure, 50% outside a seizure	1.3	1.5	Stimulation started before seizure, 50% outside a seizure	0.5	0.8
Stimulation ended a seizure	3.1	2.8	Stimulation ended a seizure	12	8.1
Stimulation right after a seizure	1.1	0.8	Stimulation right after a seizure	1.8	1.6
Seizures			Seizures		
Total number of seizures	40.2	28.3	Total number of seizures	49.6	34.6
Seizures without a stimulation	15	7.8	Seizures without a stimulation	19.8	8.2
Duration of seizures (s)	6.1	5.2	Duration of seizures (s)	5.1	4.0

Table 1.

Characteristics of the stimulations and seizures during a 45min fMRI scanning period.

Occurrences (numbers/45min) of each stimulation type during scanning period are presented, as well as occurrences and duration of seizures.

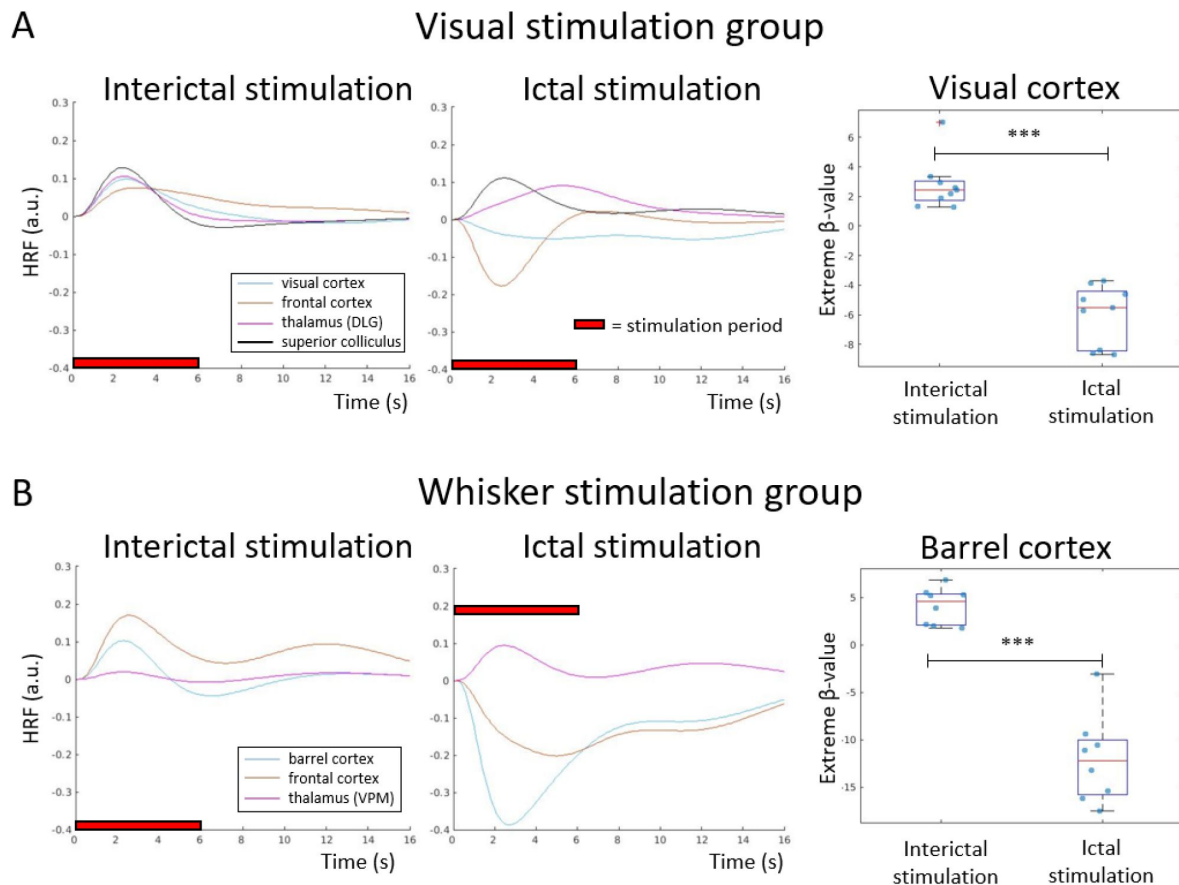


Figure 4.

Hemodynamic response functions to stimulations performed during an interictal and ictal period: visual stimulation (A) and whisker stimulation (B) groups.

HRFs were calculated by multiplying each of the three gamma basis functions with its corresponding average beta value over a selected ROI and summing them up. For statistical comparison, extreme beta-values over a ROI were calculated and values between two states were compared with a two-sample t-test. Scatter plot represents mean $\pm$ SD and each blue dots corresponds to extreme beta-values observed from individual fMRI sessions. *** = $p < 0.001$.

During a seizure, responses due to stimulations were less pronounced in the thalamus and the frontal cortex and still present in the somatosensory cortex. More voxels were activated during an interictal state compared to a seizure state (179% more). When comparing statistical responses between both states, significant changes ($p < 0.05$, cluster-level corrected) were noticed in the somatosensory, auditory and frontal cortices, with these regions being less activated in interictal state (see also [Figure 4](#)).

3.3. Analyses of brain hemodynamic response functions to sensory stimulations during ictal and interictal brain state

HRFs were calculated in ROIs in response to ictal and interictal stimulus. ROI selection was based on statistical differences seen in activation maps. Also, HRFs were calculated in response to stimulation that ended a seizure and was compared to the response when stimulation did not end a seizure ([Figure 4](#)–figure supplement 1). Extreme beta-values from between the states were compared for statistical analysis.

In the visual stimulation group, extreme beta-value of the response in the visual cortex ROI was 2.8 ± 1.7 during the baseline period, and -6.0 ± 2.0 during the seizure period, with a significant difference between the two conditions ($p < 0.001$). Interestingly, the response amplitude was higher when the stimulation ended a seizure compared to when it did not (8.1 ± 7.0 vs -6.0 ± 2.0 , $p < 0.001$). In the whisker stimulation group, the extreme beta-value of the response in the barrel cortex was 4.1 ± 1.9 during the baseline period, and -9.0 ± 1.9 during a seizure, also with a significant difference between the two conditions ($p < 0.001$). In this group, stimulation responses were also higher when the stimulation ended a seizure compared to when it did not (4.8 ± 2.9 vs -9.0 ± 1.9 , $p < 0.001$). Notably, HRFs amplitudes were both negatively and positively signed during the ictal state, depending on the brain region.

When analyzing the effect of a seizure itself, the sessions from the visual and whisker stimulation experiments were pooled together. Significant changes ($p < 0.05$, cluster-level corrected) were observed in various cortical areas, including the frontal, parietal and occipital regions ([Figure 5A](#)) totaling 895 voxels. Response amplitudes were predominantly negatively signed in the cortical regions, while they were positively signed in deeper brain regions, such as thalamus and basal ganglia ([Figure 5B](#)).

3.4 Simulations of sensory stimulation during ictal and inter-ictal periods

[Figure 6](#) shows the results of the simulations. First, neuronal activity is obtained from the AdEx mean-field for the interictal ([Figure 6A](#)) and ictal periods ([Figure 6B](#)). For a better description, the local field potential (LFP) and the membrane potential, calculated from the mean-field model during the SWD type of dynamics are shown in [Figure 6C](#). The LFP was computed using a recently developed kernel method (Tesler et al. 2022). We see that the model can capture the SWD pattern, also observed experimentally in electrophysiological measurements, and which is correlated with periods of hyper-polarization in the membrane potential. In the model the hyper-polarization is driven by a strong adaptation current and the SWD dynamics is suppressed by reducing the strength of this current. Thus, the switch between AI and SWD dynamics in our model is given by varying the strength of the adaptation current.

The time series and statistical maps of the whole brain simulations in response to stimulus, performed with the TVB platform (see Methods), are described in [Figures 6D](#) and [E](#). Stimulus was simulated in the primary visual cortex (indicated by the red-circled pixel). As we can see from the statistical maps, the propagation of the stimulus is drastically restrained during ictal periods in comparison with interictal periods. During the ictal periods the dynamics of the system is

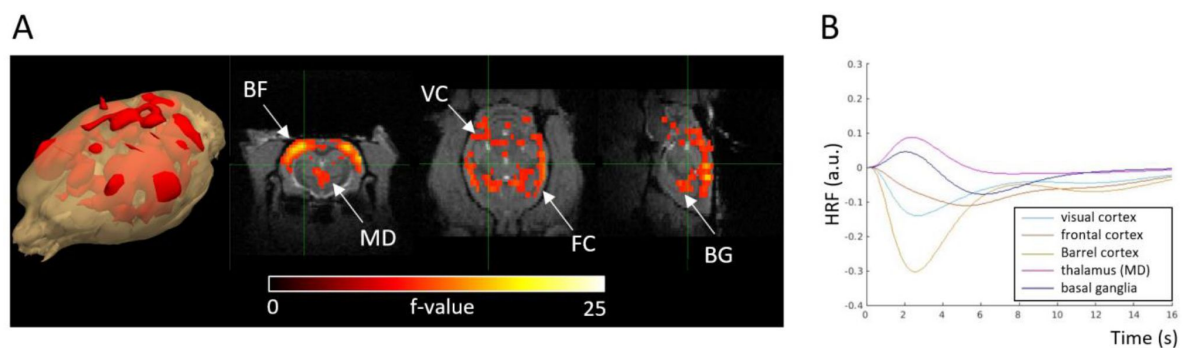


Figure 5.

Activation F-contrast map (A) and hemodynamic response functions to seizure (B).

Parameter estimates of regressors were calculated for every voxel, and contrasts were added to parameter estimates of seizure in absence of stimulation. For statistical significance, F-contrast ($p < 0.05$) maps were created and corrected for multiple comparisons by cluster level correction. HRFs were calculated by multiplying each of the three gamma basis functions with its corresponding average beta value over a selected ROI and summing them up. For both the maps and HRFs, data from visual and whisker stimulation experiments were pooled together. BF = barrel field, BG = basal ganglia, FC = frontal cortex, MD = mediodorsal thalamic nucleus, VC = visual cortex.

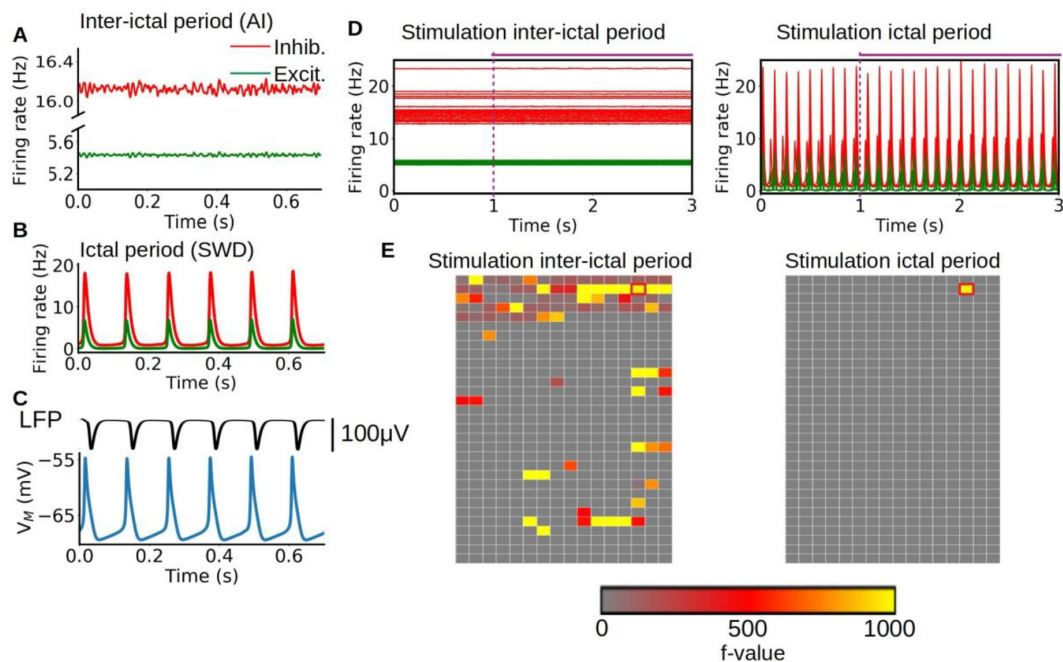


Figure 6

Simulation of sensory stimulation during ictal and interictal periods.

A-B: asynchronous irregular (AI) and spike-and-wave discharge type of dynamics obtained from the mean-field model, representing interictal and ictal periods respectively. The change between the two dynamics is given by the strength of the adaptation current in the AdEx mean-field model. C: LFP and membrane potential obtained from the mean-field model. The model can capture the SWD pattern observed experimentally in LFP measurements which is correlated with periods of hyperpolarization in the membrane potential. D-E: time-series and statistical maps of the simulated sensory stimulus in the whole brain simulations of the rat, showing the results of a stimulation of the primary visual cortex during ictal and inter-ictal periods. The onset and duration of the stimulus is indicated by the dashed vertical line and horizontal line at the top of the time-series. The statistical maps are built from a 2D representation of the 496 brain regions of the BAMS rat connectome described in the method [section 2.6](#).

dominated by the highly synchronous and regular SWD oscillation, and the effect of the stimulus does not alter significantly the ongoing dynamics. On the contrary, during the interictal periods, where the system exhibits an asynchronous irregular dynamic, the effect of stimulus generates a large variation of the ongoing dynamics in the regions linked to the stimulated area (see also Figure 6–figure supplement 1). This agrees with the reduced responsiveness observed in the fMRI results for stimulation during ictal periods.

4. Discussion

The investigation of how sensory stimulations are handled by the brain in case of absence epilepsy is essential to develop better care for individuals with this pathology and associated comorbidities. Thanks to specific fMRI and EEG recordings obtained in awake rats, we have uncovered significant differences in brain activity and activation of specific structures during visual and whisker stimulations, depending on whether they occur during a seizure period or not.

4.1. Zero echo time fMRI of non-curarized awake rats enabled absence seizure detection

Recording absence seizures in rats can be challenging because they only occur when the animals are in a calm and stress-free awake state. Despite the disturbance caused by MRI scanning noise and the need for rat restraint, we were able to successfully detect absence seizures in awake GAERS during training periods and fMRI sessions. We found that the ZTE sequence, which produced considerably lower sound pressures compared to traditional EPI, was the optimal MRI sequence for our purpose. Based on our experience from current and previous experiments, we believe that acoustic noise level is the most significant stress factor for awake rats because it can cause increased motion and thereby potential confounds in task-based and resting-state fMRI studies. ZTE-based fMRI may provide better localization of activated sites because it is sensitive to change in blood flow (Luh et al. 2000 [\[1\]](#); Restom et al. 2007 [\[2\]](#)), and it has also been suggested that ZTE-fMRI is 67% more sensitive than standard BOLD EPI due to its ability to detect increases in tissue oxygenation which shortens the T1-relaxation rate of spins in a pseudo steady-state (MacKinnon et al. 2021 [\[3\]](#)).

4.2. Whole brain responsiveness during an interictal condition

In the visual stimulation group, responses were most pronounced in the visual cortex, the superior colliculus and the thalamus, including the lateral geniculate nucleus, which are all part of the rat visual pathways (Sefton et al. 2015 [\[4\]](#)). As binocular stimulation was used, responses in both hemispheres were observed. Interestingly, we also detected activation in the frontal cortex, including the prelimbic, cingulate and secondary motor cortices. The involvement of frontal cortical areas can be explained by the fact that medial prefrontal cortex (mPFC), which includes the prelimbic cortex, is known to be a central area for rat's attention (Williams, Mohler, and Givens 1999 [\[5\]](#)) and that lesion in the mPFC impaired the accuracy of detecting brief flashes of light (Muir, Everitt, and Robbins 1996 [\[6\]](#)).

In the whisker stimulation group, responses were mostly seen in the somatosensory cortex, including the barrel field, and in the ventral posteromedial thalamus, which are part of the rat whisker system (Adibi 2019 [\[7\]](#)). This finding suggests that the somatosensory cortex, the initiating zone of the seizures, is functional during the interictal state in GAERS. Additionally, we detected activation in the frontal cortex, which could be due to increased attention to external cues. Surprisingly, activation was also observed in part of the auditory cortex. This activation could be explained by the rats' ability to differentiate the sound coming from air puffs from the ongoing MRI-noises, causing increased auditory activation during stimulation.

4.3. Decreased cortical activity during a seizure per se

Experimental and simulation results ([Figures 1](#) and [6](#)) illustrated typical synchronous and regular SWD patterns with a spike component followed by a longer-lasting wave or silence component. The silence component of SWD is thought to be caused by neuronal deactivation or increased inhibitory activity ([Fisher and Prince, 1977](#); [Gloor, 1978](#); [Avoli and Kostopoulos, 1982](#); [Inoue et al., 1993](#)), potentially resulting in overall decreased neuronal activity in the cortex. Our findings of negative HRFs in the cortical regions ([Figure 5](#)) are in line with this hypothesis, suggesting reduced neuronal activity. Supporting evidence was found in a recent neuroimaging study conducted with GAERS showing that during an absence seizure, a larger group of neural populations had a decreased firing rate, while a smaller portion of neuronal populations had an increased firing rate, leading to reduced fMRI cortical activity ([McCafferty et al. 2023](#)). Moreover, other studies utilizing optical flowmetry in GAERS have noticed decreased cerebral blood flow in cortical capillaries during an absence seizure, with preserved P_{O_2} , P_{CO_2} , and arterial blood pressure ([Nehlig et al. 1996](#)) suggesting decreased cortical brain activity. However, the mechanism for the observed subcortical fMRI increases in this study and a previous study ([McCafferty et al. 2023](#)) illustrating the disagreement between electrophysiological and fMRI signals, are yet to be explained and demand further studies.

4.4. Decreased responsiveness during an absence seizure

Based on fMRI results, we noticed reduced activation in the cortex during ictal state, along with limited propagation of activity compared to the interictal period ([Figure 4](#)), which indicates decreased responsiveness and information processing during external stimulation. Simulation results using a mean-field model also illustrated no observable change in spiking rate together with restricted propagation of neuronal activity in response to visual stimulation ([Figure 6 D](#) and [E](#), [Figure 6-figure supplement 1](#)), confirming fMRI finding. Previous electrophysiological experimental studies using whisker stimulation setups on GAERS have demonstrated that event-related potentials are modulated but still present during an absence seizure compared to seizure-free periods ([Chipaux et al. 2013](#); [Studer et al. 2019](#)). Therefore, our findings do not necessarily correspond to a decreased amplitude of event-related-potential per se but could rather indicate decreased sum of excitatory-inhibitory neuronal state when stimulating during SWD.

During a SWD, the overall neuronal activity in several cortical areas is globally decreased ([McCafferty et al. 2023](#)) potentially due to the wave component of SWD or to increased inhibition. In that state, excitation caused by external stimulation can cause relative increases of activity in responsive brain areas. However, if excitation in these neuronal populations is relatively lower compared to the neuronal suppression caused by SWD, the total sum of activity is negative compared to baseline. As fMRI is believed to measure the sum of neuronal activation ([Logothetis et al. 2001](#)), this appears as reduced fMRI amplitude, which was also apparent in measured HRFs in cortical regions ([Figure 4](#)). Thus, reduced fMRI responsiveness implies that SWD is a dominant brain feature even under stimulation condition, and that particularly the ‘wave’ phase of the neuronal oscillatory pattern of SWD can prevent responsiveness in GAERS during these conditions.

As previously discussed by Chipaux and collaborators ([Chipaux et al. 2013](#)), the lack of conscious perception to paroxysmal stimulation event could be caused by the concomitant appearance of SWD or SWD complex that is shortly (<500 ms in humans) following a stimulation. In our study, as SWD complexes co-occurred with stimulation events, this could hinder the potential conscious perception of visual and somatosensory stimulation. Interestingly, when stimulation events were applied during a seizure but were not followed by SWD complexes (*i.e.* stimulation ended a seizure), fMRI response amplitudes were higher than when stimulation did not end a seizure. This could mean that the stimulation response exceeded a threshold to shift the brain back to a responsive or conscious state, which was apparent in the fMRI signal as well. The reason for the change in brain state in these stimulation cases is unclear, as stimulation strength remained the

same throughout the experiment. However, there is a potential for future experiments and therapeutic interventions to study further how different stimulation schemes affect neuronal populations and cause a shift from ictal to non-ictal state.

5. Conclusion

In this study, we were able to detect spontaneous absence seizures inside the magnet using awake, non-curarized, rats and a quiet ZTE imaging sequence, and thereby able to study whole brain sensory perception in ictal and interictal brain states. Rats were stimulated with blue led light and with somatosensory whisker air-puffs during both interictal and ictal states. We found that cortical activation was spatially wider and stronger during interictal stimulation compared to ictal stimulation. Altogether, these stimulation-based fMRI experiments, combined with the brain-wide simulation results, suggest that rats with absence epilepsy have hindered and restricted sensory processing during an ongoing seizure, although not fully abolished. These findings may contribute to our understanding of cognitive impairments observed in patients suffering from absence epilepsy and the severe neuropsychiatric comorbidities observed in children. Additionally, the results of this study provide insight into the information processing during SWD and may aid in the development of future therapeutic approaches for absence epilepsy.

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Data Availability Statement

All raw and preprocessed data, fMRI preprocessing scripts, and scripts used for the simulations are available in Zenodo repository: <https://doi.org/10.5281/zenodo.8104455> .

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Reviewer #1 (Public Review):

Summary:

In this paper, the effects of two sensory stimuli (visual and somatosensory) on fMRI responsiveness during absence seizures were investigated in GEARS rats with concurrent EEG recordings. SPM analysis of fMRI showed a significant reduction in whole-brain responsiveness during the ictal period compared to the interictal period under both stimuli, and this phenomenon was replicated in a structurally constrained whole-brain computational model of rat brains.

The conclusion of this paper is that whole-brain responsiveness to both sensory stimuli is inhibited and spatially impeded during seizures.

I also suggest the manuscript should be written in a way that is more accessible to readers who are less familiar with animal experiments. In addition, the implementation and interpretation of brain simulations need to be more careful and clear.

Strengths:

1. ZTE imaging sequence was selected over traditional EPI sequence as the optimal way to perform fMRI experiments during absence seizures.
2. A detailed classification of stimulation periods is achieved based on the relative position in time of the stimulation period with respect to the brain state.
3. A whole-brain model embedded with a realistic rat connectome is simulated on the TVB platform to replicate fMRI observations.

Weaknesses:

1. The analysis in this paper does not directly answer the scientific question posed by the authors, which is to explore the mechanisms of the reduced brain responsiveness to external stimuli during absence seizures (in terms of altered information processing), but merely characterizes the spatial involvement of such reduced responsiveness. The same holds for the use of mean-field modeling, which merely reproduces experimental results without explaining them mechanistically as what the authors have claimed at the head of the paper.
2. The implementations of brain simulations need to be more specific.

Contribution:

The contribution of this paper is performing fMRI experiments under a rare condition that could provide fresh knowledge in the imaging field regarding the brain's responsiveness to environmental stimuli during absence seizures.

Reviewer #2 (Public Review):

Summary:

This study examined the possible effect of spike-wave discharges (SWDs) on the response to visual or somatosensory stimulation using fMRI and EEG. This is a significant topic because SWDs often are called seizures and because there is non-responsiveness at this time, it would be logical that responses to sensory stimulation are reduced. On the other hand, in rodents with SWDs, sensory stimulation (a noise, for example) often terminates the SWD/seizure.

In humans, these periods of SWDs are due to thalamocortical oscillations. A certain percentage of the normal population can have SWDs in response to photic stimulation at specific frequencies. Other individuals develop SWDs without stimulation. They disrupt

consciousness. Individuals have an absent look, or "absence", which is called absence epilepsy.

The authors use a rat model to study the responses to stimulation of the visual or somatosensory systems during and in between SWDs. They report that the response to stimulation is reduced during the SWDs. While some data show this nicely, the authors also report on lines 396-8 "When comparing statistical responses between both states, significant changes ($p < 0.05$, cluster-) were noticed in somatosensory auditory frontal..., with these regions being less activated in interictal state (see also Figure 4). That statement is at odds with their conclusion.

They also conclude that stimulation slows the pathways activated by the stimulus. I do not see any data proving this. It would require repeated assessments of the pathways in time.

The authors also study the hemodynamic response function (HRF) and it is not clear what conclusions can be made from the data.

Finally, the authors use a model to analyze the data. This model is novel and while that is a strength, its validation is unclear. The conclusion is that the modeling supports the conclusions of the study, which is useful.

Strengths:

Use of fMRI and EEG to study SWDs in rats.

Weaknesses:

Several aspects of the Methods and Results are unclear.

Reviewer #3 (Public Review):

Summary:

This is an interesting paper investigating fMRI changes during sensory (visual, tactile) stimulation and absence seizures in the GAERS model. The results are potentially important for the field and do suggest that sensory stimulation may not activate brain regions normally during absence seizures. However the findings are limited by substantial methodological issues that do not enable fMRI signals related to absence seizures to be fully disentangled from fMRI signals related to the sensory stimuli.

Strengths:

Investigating fMRI brain responses to sensory stimuli during absence seizures in an animal model is a novel approach with the potential to yield important insights.

The use of an awake, habituated model is a valid and potentially powerful approach.

Weaknesses:

The major difficulty with interpreting the results of this study is that the duration of the visual and auditory stimuli was 6 seconds, which is very close to the mean seizure duration per Table 1. Therefore the HRF model looking at fMRI responses to visual or auditory stimuli occurring during seizures was simultaneously weighting both seizure activity and the sensory (visual or auditory) stimuli over the same time intervals on average. The resulting maps and time courses claiming to show fMRI changes from visual or auditory stimulation during seizures will therefore in reality contain some mix of both sensory stimulation-related signals and seizure-related signals. The main claim that the sensory stimuli do not elicit the same activations during seizures as they do in the interictal period may still be true. However the attempts to localize these differences in space or time will be contaminated by the seizure-related signals.

The claims that differences were observed for example between visual cortex and superior colliculus signals with visual stim during seizures vs. interictal are unconvincing due to the above.

The maps shown in Figure 3 do not show clear changes in the areas claimed to be involved.