

Sepsis modulates cortical excitability and alters the local and systemic hemodynamic response to seizures

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Abstract

Non-convulsive seizures and status epilepticus are frequent and associated with increased mortality in septic patients. However, the mechanism through which seizures impact outcome in these patients is unclear. As previous studies yielded an alteration of neurovascular coupling (NVC) during sepsis, we hypothesized that non-convulsive seizures, might further impair NVC, leading to brain tissue hypoxia and to secondary brain injury. In an ovine model, seizures were induced by cortical application of penicillin. Animals were allocated to sham procedure or sepsis; septic animals were studied either during the hyperdynamic phase (sepsis group) or after septic shock occurrence (septic shock group). We recorded a greater seizure-induced increase in the EEG gamma power in the sepsis group than in sham. Using a neural mass model, we also showed that the activity of the inhibitory interneurons, crucial to induce gamma oscillations, were relatively greater in the sepsis group. However, the NVC was impaired in sepsis animals, despite a normal brain tissue oxygenation. In septic shock animals, it was not possible to induce seizures. Cortical activity declined in case of septic shock, but it did not differ between sham or sepsis animals. As the alteration in NVC preceded cortical activity reduction, we suggest that, during sepsis progression, the NVC inefficiency could be partially responsible for the alteration of brain function, which might prevent seizure occurrence during septic shock. Moreover, we showed that cardiac output decreased during seizures in sepsis animals instead of increasing as in shams. The alteration of the seizure-induced systemic hemodynamic variations in sepsis might further affect cerebrovascular response to neuronal activation. Our findings support the hypothesis that anomalies in the cerebral blood flow regulation may contribute to the sepsis-associated encephalopathy and that seizures might be dangerous in such a vulnerable setting.

Introduction

Critically ill patients with sepsis may sometimes experience non-convulsive seizure (NCSz) and non-convulsive status epilepticus (NCSE) ^{1,2}. Some studies also reported an association between the occurrence of seizure or periodic epileptiform discharges and increased mortality ^{1,2} in patients with sepsis; however, the mechanisms through which NCSz and NCSE might impact outcome in this setting have not been elucidated. Also, infections and sepsis are frequent and associated with worse outcome in patients admitted to an intensive care unit (ICU) with NCSE ³, suggesting a significant interplay between the infectious and epileptic events on mortality.

In brain-injured patients, NCSz are associated with cerebral hypoxia and metabolic stress, in part due to alteration in neurovascular coupling (NVC) ^{4,5}. NVC refers to the compensatory mechanisms that adjusts cerebral blood flow (CBF) to neuronal activity and metabolic demand to different stimuli ⁶. Human and animal studies have found an impairment of NVC during sepsis, both for spontaneous ⁷ or stimulus-induced cortical neuronal activation ⁶. As NCSz consist of increased hypersynchronous neuronal activity and metabolic demand ⁸, it is thus conceivable that they might contribute to exceed NVC function, potentially resulting in brain tissue hypoxia and secondary injury during sepsis.

In this study, we aimed therefore to assess whether NCSz lead to cerebral hypoxia in an ovine model of sepsis due to an abnormal NVC. Since sepsis is a gradual process with variable severity of organ dysfunction ⁹, we also sought to compare the effects of NCSz during both isolated hyperdynamic phase without cardiovascular impairment and shock. We hypothesized that NVC would be more altered during shock and this might be associated with a more deleterious effect of NCSz on tissue oxygenation. We also evaluated the systemic effect of NCSz and the sepsis-induced alteration in cortical excitability in this setting.

Results

Parameters at time of seizure induction and seizure induction.

Seventeen female sheep were included in the final analysis (6 in the sham, 5 in the sepsis and 6 in the septic shock group); one animal was excluded from the sepsis group, as it was found to be already septic at the time of delivery. A summary of systemic, biological parameters and information at the time of penicillin application is presented in Table 1. Except for MAP, which was lower in the septic shock group, groups did not show any significant difference concerning variables that could affect CBFv estimation or neuronal excitability, such as hemoglobin concentration, temperature, pH, PaO₂ or PaCO₂. The fast/delta ratio calculated before

penicillin application was statistically lower in the septic shock group than the sham and sepsis groups, but did not differ between the sham and sepsis groups (Table 1).

Table 1. Summary of systemic, biological parameters and information at time of penicillin application in the studied animals

Parameter	Sham				Sepsis				Septic shock				q values						
	Median [IQR]				Median [IQR]				Median [IQR]										
CI_std (L/min/m ²)	1.2	[0.9	-	1.3]	1.6	[1.5	-	1.9]	1.5	[1.3	-	1.6]	0.07
MAP (mmHg)	91 ^c	[83	-	105]	77 ^c	[77	-	90]	62	[54	-	65]	0.02
HR (bpm)	102	[96	-	109]	130	[130	-	153]	146	[133	-	152]	0.07
MPAP (mmHg)	12.6	[9	-	13]	7.7	[7	-	9]	11	[11	-	21]	0.08
PaCO ₂ (mmHg)	34.5	[34	-	36]	35.6	[34	-	36]	35.8	[34.8	-	39.1]	0.69
PaO ₂ (mmHg)	118	[117	-	120]	110	[108	-	114]	90	[74	-	107]	0.07
pH	7.44	[7.40	-	7.46]	7.44	[7.39	-	7.47]	7.31	[7.28	-	7.36]	0.07
Lactate (mmol/l)	0	[0	-	0]	1	[1	-	1.6]	2	[1.75	-	2.74]	0.07
Hb (g/dl)	8.3	[8.2	-	9.3]	9.4	[8.5	-	10.0]	8.8	[8.4	-	9.5]	0.64
T (°C)	40.0	[39.0	-	41.0]	39.5	[39.3	-	40.1]	40.6	[40.3	-	40.7]	0.24
PaO ₂ /FiO ₂	385	[337	-	399]	378	[327	-	383]	191	[102	-	240]	0.07
Weight (kg)	29.5	[26.8	-	30]	29.5	[26	-	33]	24.5	[24	-	25.8]	0.24
Latency to penicillin application (h)	6.2	[6.0	-	12.0]	8.5	[8.1	-	9.0]	12.6	[11.2	-	13.7]	0.14
Total penicillin dose (UI) x10 ⁴	115	[85	-	145]	100	[100	-	110]	85	[50	-	97.5]	0.24
Latency to the 1st seizure (min)	118	[75	-	142]	81	[53	-	131]	NA	[NA	-	NA]	0.62
Number of induced seizures	50	[27	-	77]	37	[32	-	47]	NA	[NA	-	NA]	0.78
Period of observation (h)	6.0	[5.5	-	7.0]	6.3	[4.7	-	9.7]	1.8	[1.3	-	2.8]	0.07
Cumulative MDZ dose (mg/kg)	7.3	[6.7	-	8.1]	10.3	[9.9	-	10.4]	8.7	[8.2	-	11.9]	0.64
Cumulative KET dose (mg/kg)	48.7	[45.0	-	54.3]	68.6	[65.7	-	69.5]	57.7	[54.5	-	79.2]	0.64
Fast/delta ratio	2.3 ^c	[1.6	-	3.4]	2.3 ^c	[1.3	-	2.9]	1.3	[1.1	-	2]	< 0.01

Data are presented as median and (IQR). Abbreviations: CI_std: cardiac index standardized, expressed as variation of the cardiac index of the beginning of the experiment; MAP: mean arterial pressure; HR: heart rate; MPAP, mean pulmonary arterial pressure; Hb:

hemoglobin; T °C: temperature in Celsius degrees; PaO₂/FiO₂: ratio used to identify and quantify acute lung injury (acute lung injury if ratio ≤ 300, acute respiratory distress syndrome if ratio ≤ 200); PD, periodic discharges. MDZ: midazolam; KET: ketamine; NA, not applicable. Fast-delta ratio of the ECOG signal was calculated before penicillin application. Variables were analyzed using the Kruskal-Wallis test. P-values were corrected for false discovery rate (q-values); the Dunn's post hoc tests are presented in case of q-values < 0.05 (in bold in the table). ^C: significant at 5% vs. septic shock.

We observed no difference in the latency to the first seizure nor in the total number of seizures per animal between the sham and early sepsis groups (p = 0.88 and p = 0.83 respectively). Although the total dose of applied penicillin did not differ between groups, we were not able to induce seizures in the septic shock group. Of note, the total observation time was shorter, albeit non-significantly, in the septic shock group, since spontaneous death occurred. On the other hand, there were no differences between the total observed period in the septic shock group and the latency to the first seizure in other animals (p = 0.57).

Cerebral parameters variations during seizures.

Figure 1, panel a shows the ictal variations of local and systemic parameters. In the sepsis group, the increase in the E_γ was greater throughout all the duration of the seizures than in the sham group. On the other hand, the ictal increase in the cerebrovascular parameters did not differ between the two groups, albeit the CBF_v variation lasted longer in the septic animals. In the sepsis group, the AUC of ictal variations of both E_γ and CBF_v was greater than in the sham group (P < 0.01, for both, **Fig. 1, panel b**). The variations in E_γ and CBF_v were positively correlated in both groups (Spearman's coefficient ρ = 0.4, P = 0.02 for shams & ρ = 0.44, P = 0.02 for sepsis, **Supplementary material, Table S1**). The E_γ /CBF_v ratio was greater in the sepsis group (P = 0.002, **Fig. 1, panel b**). The positive ictal variation of the PbtO₂ did not differ between groups (P = 0.66) but the post-ictal decrease in PbtO₂ was both longer and deeper in the sham than in the sepsis group (p = 0.02). There were no statistically significant differences in the peak lags between groups (**Supplementary material, Table S2**). The temporal evolution of synaptic gains is shown in **Fig. 2**. All the gains decreased during seizures in both groups but, in the sepsis group, a delayed onset of the variation in all the considered parameters was observed. Accordingly, the lag to the peak of the negative variation tended to be delayed, albeit not significantly (P = 0.67 for excitatory, and P = 0.19 for the fast inhibitory synaptic gain, **Supplementary material, Table S3**). The AUC of the synaptic gains tended to be lower in sepsis animals (**Supplementary material, Fig. S4 and Table S3**), but only for the fast inhibitory synaptic gain, G, the difference was significant (P = 0.001).

Systemic hemodynamic parameters during seizures.

In the sham group, after seizure onset, the increase in MAP was accompanied by a comparable increase in the HR and a slight rise in the CO (**Fig. 3**). The CO_{EST} and HR changes in the sepsis group showed an opposite pattern, since MAP increase coincided with a reduction of the CO_{EST} and of the HR. Furthermore, ictal MAP variation was greater in the sepsis group (8% [-6.3–10.4] vs 2.5% [-2.6–3.8]; p = < 0.01).

Discussion

In this work, we show that sepsis influences both cerebral and systemic responses to NCSz. The enhanced sepsis-induced EEG power during seizure is accompanied by a reduced NVC response.

The microcirculatory changes induced by sepsis, such as the reduction in the proportion of cortical perfused vessels¹⁰, the astrocyte end-feet¹¹ and pericytes¹² detachment from the vessel walls and increased the blood-brain-barrier permeability¹³ could alter the extracellular milieu of the brain, interfering with the neurovascular unit. Moreover, release of cytokines with a high vasoconstriction power, such as endothelin-1¹⁴, might move the physiological balance towards arteriolar vasoconstriction, either directly or by a reduction in endothelial nitric oxide production¹⁵. Consistent with these hypotheses, we showed that the seizure-induced cerebral vascular response in the sepsis group is relatively flattened. In fact, despite a more important neuronal and vascular activation, the E_γ/CBF_v ratio was greater in the sepsis group, suggesting that the increase in neuronal activity was associated with a proportionally smaller CBF_v variation than in shams. Possibly because of this reduced vascular response to neuronal activation, the rise in the PbtO₂ tended to be delayed. These findings agree with previous studies that suggested that sepsis alters but not completely abolishes the NVC^{16,17}. Indeed, we showed a persistent positive correlation between neuronal

activation and vascular response in both groups. As sepsis progresses, more severe impairment of NVC, as previously demonstrated¹⁸ could be partially responsible for the alteration of brain function, which might prevent seizure occurrence, as observed in this study.

Interestingly, the altered NVC does not translate into an immediate neuronal dysfunction or substantial tissue hypoxia. Auxiliary sources of energy than mitochondrial oxidative respiration, such lactate or ketone bodies, might thus support neuronal activity^{19,20} in the context of reduced O₂ availability. In line with this hypothesis, the physiological PbtO₂ dip after seizure offset²¹ was relatively smaller in sepsis animals, suggesting a reduced O₂ consumption. Since we did not find a reduction in cortical activity in sepsis animals (Table 1), we deemed less likely that the smaller changes in the PbtO₂ would be due to a reduced cortical metabolic demand, as it has been shown to occur in sepsis²². As sepsis progresses, this cerebrovascular inefficiency could be partially responsible for the alteration of brain function, which might prevent seizure occurrence.

Animal studies have shown that systemic inflammation enhances cerebral excitability, as revealed by an increase in seizure susceptibility²³. Innate immune cells (i.e. microglia) could play a major role in this pathological response²⁴ and previous studies have demonstrated microglial activation in sepsis²⁵⁻²⁷. Moreover, some human studies suggested that sepsis was a significant risk factor for NCSz¹ and probably for NCSE². In line with them, an increase of seizure susceptibility would have been expected in the sepsis groups; instead, although a prominent ictal Ey power in sepsis animals, no seizures were observed in the septic shock group, despite a similar penicillin dose. In severe septic animals, brain function and perfusion could be altered to such an extent to reduce the ability of neurons to fire synchronously and thus prevent seizure occurrence, as suggested in a human study²⁸. Using the fast/delta frequency ratio as a surrogate for cortical neuronal activity²⁹, we showed that septic shock animals presented a lower ratio than sham or sepsis group, suggesting an impairment of neuronal function. Brain dysfunction is a well-known complication of sepsis (i.e. sepsis-associated encephalopathy - SAE)³⁰ and it has been shown that increasing severity of brain dysfunction was associated with progressing slowing of brain activity³¹, resulting in a decrease in the fast/delta ratio. The presence of a sepsis-induced NVC alteration, as we have shown with the reduced hemodynamic response to the increase in Ey power in sepsis animals, might play a crucial role in SAE development. In fact, as the fast/delta ratio was not statistically different between sham and sepsis animals, our data support the hypothesis that, in sepsis, NVC impairment precedes neuronal dysfunction⁶, which successively develops when septic shock occurs¹⁸. Since no seizure occurred in septic shock animals, we were not able to test the NVC in this scenario and in particular to answer the question of whether seizures are associated with a reduction in tissue oxygenation during septic shock.

We used the NMM to estimate if a difference in temporal evolution of the synaptic activity of the modeled neuronal populations might explain the differences in neuronal activation and in the cerebrovascular hemodynamic response in septic animals. It has been shown that sepsis induces variations in important neurotransmitter metabolisms, such as those of acetylcholine³², noradrenaline³³ and gamma-aminobutyric acid (GABA)³⁴, and this might play a role in SAE. For all the analyzed parameters, we noticed a deflection during seizure. These alterations are consistent with what was previously reported in the literature³⁵ and they suggest an excitation/inhibition imbalance leading to seizure. Of note, the variations were delayed and blunted in the sepsis group, suggesting a certain extent of synaptic impairment induced by sepsis which might not be homogenous between neuronal populations as it happens for ischemic insults³⁶. Therefore, the activity of the fast somatic-projecting inhibitory interneurons, which are described by the fast inhibitory synaptic gain G in the NMM model, was less reduced in the sepsis group. Since these interneurons play a crucial role in inducing gamma oscillations³⁷, this finding might partially explain the more prominent Ey power in sepsis. Furthermore, we may speculate that the delayed activation of neural populations in sepsis might explain the delayed seizure-induced peak in CBFv increase we recorded. Finally, these excitation/inhibition imbalance might furtherly worsen as sepsis progress, contributing to the negative influence of an altered NVC in preventing seizure occurrence in septic shock.

The ictal systemic hemodynamic response differences between groups are intriguing but hard to explain. Although cardiovascular effects of NCSz are a matter of debate, sympathetic outflow seems to be enhanced during seizures³⁸. The systemic cardiovascular response in the sham group reflects the physiologic effect of sympathetic stimulation leading to a slight increase in MAP accompanied by a chrono- and inotropic cardiac effect³⁹. In the sepsis group, an increased α adrenergic receptor (AR) activity⁴⁰ might mediate a rise in MAP by systemic vasoconstriction, inducing a baroreceptor reflex activation and, thus, a reduction in HR

and CO_{EST} . Moreover, an adrenal insufficiency, which has been reported in sepsis⁴¹, may reduce the positive cardiac adrenergic effect of adrenaline; the rise in BP would no longer be combined with a positive chronotropic effect but by a negative one mediated by baroreceptor reflex. Data interpretation might be further complicated by the interaction of other mechanisms influencing cardiovascular physiology, such as arginine-vasopressin (AVP) and the renin-angiotensin systems (RAS). It has been shown that they share superimposable inotropic and peripheral-vasoconstriction effects in healthy subjects^{42,43}. Therefore, despite an increase in AVP has been reported after convulsive seizures⁴⁴, it is challenging to establish if the rise in plasma concentration is a direct effect of seizure or it is an epiphenomena of the physiologic anomalies accompanying convulsion (i.e. hypotension, hypoxia, autonomic nervous system activation⁴⁵). Moreover, the role of these hormones in non-convulsive seizure is unknown. Several studies has shown that sepsis induces a depletion of plasmatic levels of AVP and a downregulation of angiotensin receptors in vascular bed^{46,47}. It is thus conceivable that the sepsis-induced alteration in these physiologic systems might influence the cardiovascular response to seizure that we recorded in sepsis animals. Further research is needed to explore the ictal variations of AVP and RAS in non-convulsive seizures.

Previous studies have essentially focused on local cerebral mechanisms of CBF regulation, especially NVC, to describe the vascular response to seizure^{48,49}. However, evidence from both humans and animals experiments suggests a close relationship between CO and CBF⁵⁰ and a study in septic patients showed a linear relationship between cardiac index and cerebral blood flow⁵¹. Despite the difficulty to distinguish the net effects of cerebral and systemic factors influencing CBF, we may speculate that the alteration in systemic hemodynamic recorded in sepsis animal, especially the reduction in the ictal CO_{EST} , might negatively influence the physiologic CBF response to neuronal activation.

Our study presents several limitations. First, regarding the latency to seizure onset in septic shock group, one could speculate that, since a neuronal dysfunction occurs in severe sepsis, the time necessary to induce seizure in the septic shock group could have taken longer than in other groups. Since specific sepsis therapies, such as antibiotics, might enhance seizure excitability⁵², we preferred not to employ them; thus, this hypothesis could not be verified since septic shock animals spontaneously succumbed. However, human data seem to support our presumption that severe sepsis-induced cerebral dysfunction prevents seizure occurrence²⁸. Secondly, the variations in tissue oxygenation we recorded are smaller than those reported in other animal studies^{48,49}. Our data might be the average of the ictal focus and surrounding tissue $PbtO_2$ variations, which are not identical and, in fact, often opposite. Furthermore, since we did not measure any metabolic parameters, we could not definitely conclude to a reduced oxygenation consumption. Of note, no specific intra-group variations or inter-groups differences in $PbtO_2$ values were recorded during resting-state conditions. Thirdly, NMM privileges the description of key mechanisms of neuronal networks using simplified assumptions and empirical priors⁵³, which has been successful in reproducing EEG signals in patients⁵⁴. Thus, it is possible that the set of parameters fitting humans may not be appropriate for our experimental model. Finally, the influence of cerebral autoregulation (CA), defined as the capacity of the brain to maintain an adequate cerebral blood flow despite variations in cerebral perfusion pressure⁵⁵, could be considered as a confounding factor in the interpretation of the link between systemic and local variables. As sepsis and seizures seem to alter CA^{56,57}, CBFv variations could be influenced by MAP fluctuations rather than by the sepsis-induced NVC alteration. Moreover, since we did not find significant differences in the correlation between EEG/CBFv ratios and ictal-induced CO_{EST} variations between groups, the negative influence of the reduction in the ictal CO_{EST} on the CBF response to neuronal activation remains a matter of speculation.

Conclusions

In conclusion, our study shows that sepsis alters both local and systemic responses to seizures. The NVC is blunted in sepsis, contributing to the alteration in brain function that develops as sepsis progress, and possibly preventing seizure occurrence in septic shock. The alteration of the systemic hemodynamic support to cerebral perfusion might affect cerebrovascular response to neuronal activation. These anomalies probably contribute to the sepsis-associated encephalopathy.

Methods

General procedure.

Animal model procedures have been previously described^{18,58}. Briefly, the Institutional Review Board for Animal Care of the Free University of Brussels (Belgium) approved all experimental procedures (number of Ethical Committee approval: 675N), which were also in compliance with ARRIVE (Animal Research: Reporting in Vivo Experiments) guidelines. Care and handling of the animals were in accord with National Institutes of Health guidelines (Institute of Laboratory Animal Resources). The protocol was performed on 18 female *Ovis Aries* sheep. Animals were allocated with a 1:1:1 ratio to a sham procedure (n = 6), early sepsis assessment (n = 6) and septic shock assessment (n = 6) groups. Sample sizes were based on previous studies from our laboratory using the same animal model⁵⁸. After randomization, animals were excluded if they presented a hemoglobin level below 8 g/dL or systemic signs of infection at time of delivery to the laboratory. Left-side craniotomy was performed using a high-speed drill (Wuhu Ruijin Medical instrument, Wuhu, China) and a 2.5 cm² hole was opened in the frontal-parietal bone using a laminectomy tool (Aesculap-WerkeAG, Tuttlingen, Germany). The dura mater was opened with scissors and a 4-contact electrocorticography (ECOG) electrode (Dixi Medical, Besançon, France) was slipped beneath the dura over the cortex surface of the post-central gyrus and taped to the skull. Near the ECOG electrode, the dura mater was subsequently punctured to insert a laser-Doppler flowmetry probe (OxyFlow 4000, Oxford Optronix, UK) for local cerebral blood flow velocity (CBFv) measurement and a Clark electrode (Licox; Integra Lifesciences, Zaventem, Belgium) for brain tissue oxygen partial pressure (PbtO₂) measurement. All catheters were placed under sterile conditions at a depth of 0.5 cm into the brain parenchyma as close as possible to each other. Except for sham animals, feces were collected from the caecum through a midline laparotomy (1.5 g.kg⁻¹ of body weight). A plastic tube was inserted through the laparotomy incision in the abdominal cavity for successive feces injection.

Monitoring and Measurements.

Monitoring protocol has been previously published^{18,58}. Briefly, a continuous IV infusion of ketamine, morphine, and midazolam was used as general anesthesia throughout the entire experiment adjusting initial doses to achieve a nearly continuous EEG background⁵⁹. Ventilator parameters were adjusted to maintain PaO₂ and PaCO₂ values in the normal ranges. Mean arterial pressure (SC 9000 monitor; Siemens, Berlin, Germany), heart rate (HR), core-temperature and cardiac output (CO) (Vigilance II monitor; Edwards Lifesciences, Irvine, California, United-States) were monitored continuously. Systemic hemodynamic parameters, ECOG, PbtO₂, and CBFv were recorded continuously and simultaneously with a sampling rate of 250Hz (Notocord-hem, Instern Company, France). Measurements of mean pulmonary arterial pressure were collected every 1.5 h. Cardiac index (CI) was calculated using standard formulas; the body surface area was estimated from Mitchell's sheep-specific formula⁶⁰.

Experimental Protocol.

After surgical procedures, the animal was allowed to stabilize. In all groups, balanced crystalloid solution (Plasmalyte; Baxter, Deerfield, Illinois, USA) and 6% hydroxyethyl starch solution (Voluven; Fresenius Kabi, Schelle, Belgium) were titrated to prevent and treat hypovolemia, detected by integrating a reduction in mean arterial pressure (i.e. >10% from baseline), cardiac and urinary outputs (> 10% from baseline and < 0.5cc/kg*h, respectively) and/or mixed venous blood oxygen saturation (< 70%). In all septic animals, feces were injected into the abdominal cavity. Seizures (see below for seizure definition) were induced by topical application of penicillin G on the cerebral cortex beneath the left-sided ECOG electrode; 10⁵ penicillin UI, diluted in 0.1 mL of CNS perfusion fluid (Perfusion Fluid CNS, CMA Microdialysis AB, Sweden), were applied every 15 minutes until seizures were induced (**Supplementary material, Fig. S1**). Of note, since animals were paralyzed, seizures were all non-convulsive. In the sepsis group, penicillin application occurred during the hyperdynamic phase of sepsis, arbitrarily defined when the cardiac index (CI) exceeded by 130% and the pulse pressure (PP) by 140% their baseline values. At the end of the experiment, animals were sacrificed with a bolus of IV potassium chloride. In the septic shock group, penicillin was applied after the onset of shock, defined, according to the last consensus definitions⁹, as MAP < 65 mmHg persisting more than 15 minutes and lactate elevation > 2 mmol/L despite adequate fluid resuscitation. Data were collected until spontaneous death occurred.

Study Outcomes.

The primary outcome of the work was to study the effects of sepsis on NVC during non-convulsive seizures. Our hypothesis was that non-convulsive seizures, occurring during sepsis, might further impair NVC and result into brain tissue hypoxia. The second aims of the work were to assess systemic hemodynamic responses to seizures and to analyze the influence of sepsis on cortical excitability.

Data analysis.

Cerebral and systemic parameters were analyzed as previously described⁵⁸. All analyses were performed off-line, using built-in and custom functions in Matlab (The MathWorks, Natick, MA, USA). Physiologically implausible measures and outliers, defined according to Chauvenet's criteria⁶¹, were removed. Seizure onsets were defined as rhythmic discharges with a frequency ≥ 3 Hz, according to consensus criteria⁵⁹; seizure offsets corresponded to post-ictal ECOG signal attenuation or when discharge frequency dropped below 3 Hz. For each detected seizure, ECOG epochs from 30 s before to 60 s after seizure onset were selected (**Supplementary material, Fig. S2**); if the inter-ictal interval between two consecutive seizures was shorter than 60 s, both seizures were discarded. The envelope of the low gamma frequency band (30–50 Hz) (Ey) was extracted from the ECOG signal using wavelet transform spectral density estimate (Morse wavelet; *cwt*, *icwt* and *envelope* functions in Matlab). Corresponding physiological variables (MAP, HR, CBFv, PbtO₂ and CO) were considered; since the sampling frequency of the CO monitor was too low to identify rapid variations, the Liljestrand-Zander method⁶² was employed to estimate CO from the pulse waveform (CO_{EST}). CBFv signal was high-pass filtered with a cut-off frequency of 0.25 Hz. Linear interpolation was used to replace excluded data. Finally, physiological variables were expressed as percentage variations from baseline using the mean calculated on the pre-ictal 15 s period, as previously described⁶³, and their value set to 0 at seizure onset; for Ey, baseline values were simply set to zero. Local (CBFv and PbtO₂) and systemic (MAP, HR, CO_{EST}) parameters were compared between groups, taking the first 8 analyzable seizures for each animal of each group, in order to have a substantial sample size for statistical analysis thus limiting the number of animals needed. For each parameter, the value of the peak of the ictal maximal absolute deviation from the pre-ictal baseline, its lag from the seizure onset and the area under the curve (AUC) of its variation were considered. To assess the NVC response, we compared the ratio of the AUC of the Ey and that of the CBFv between groups.

We analyzed the evolution of EEG activity during seizures using a neural mass model (NMM), which was shown to accurately reproduce real EEG signals⁵⁴. NMM provides mathematical description of neuronal oscillation and EEG rhythms by modeling average properties and interactions of a large numbers of neurons, i.e. cortical pyramidal cells and interneurons. Previous studies suggested that two classes of inhibitory interneurons, slow dendritic-projecting inhibitory interneurons and fast somatic-projecting inhibitory interneurons, play a role in formation of EEG rhythms⁶⁴. In particular fast-spiking interneurons were crucial in inducing gamma oscillations³⁷, which are one of the most characteristic intracranial EEG pattern in epileptic seizures. These evidences were successfully implemented in a NMM of epilepsy in humans^{35,54} based on four neuronal population subgroups (main population of pyramidal cells, excitatory interneurons, slow inhibitory and fast inhibitory interneurons). The NMM was automatically fitted to EEG to analyze the temporal evolution (between 30 s before to 60 s after seizure onset) of three meaningful parameters - the average excitatory (Ae), the slow and fast inhibitory synaptic gains (B and G) – as previously proposed^{35,54,65,66}. We calculated the AUC and the lag from the beginning of the seizure to the peak of the variation for each parameter.

Spectrograms were calculated using the wavelet transform spectral density estimate (Morse wavelet; *cwt* function in Matlab). To represent the integrity of cortical neuronal activity, the fast-delta ratio (FDR) was further calculated, as the ratio between the sum of the power in the gamma (15–100 Hz) and beta (8–14 Hz) frequency bands divided for the power of the delta (0.1–4 Hz) ones⁷.

Statistical Analysis.

Statistical analyses were performed using Matlab (MathWorks) and SPSS for Windows (IBM SPSS Statistics 25, Chicago, IL). A p value < 0.05 was considered statistically significant. Data are presented as mean \pm SD or median and [IQR]. Kruskal-Wallis test was used to assess differences between groups. The linear step-up procedure introduced by Benjamini and Hochberg was applied for controlling the false discovery rate (q-value)⁶⁷. Dunn's post-hoc analysis was employed in case of a q-value < 0.05 . To compare ictal-induced local and systemic parameters changes between groups, point by point repeated t-tests were performed (*ttest2* function in Matlab).

The AUC, the peak amplitude and the lag of the ictal cerebral parameters variations from the baseline were also compared between groups using a Wilcoxon test. The Spearman's rank-order correlation coefficient was used to measure the strength of the association of the ictal variations of Ey and those of PbtO₂ and CBFv. In fact, previous studies showed a positive linear correlation between epileptic neuronal activity and the amplitude and the duration of cerebrovascular parameters⁶⁸. To compare the vascular

response to neuronal activation of sham and sepsis groups, we used the ratio between the AUC of the Ey and that of the CBFv, which was supposed to remain stable over time. Synaptic excitatory (Ae) and inhibitory (G and B) gain variations during seizures were compared, point by point, between the sham and sepsis groups with the corresponding gains in septic animals using a repeated t-test (*ttest2* function in Matlab).

To ensure that all measured parameters were stable over time before seizure induction, 8 consecutive 90 seconds periods were selected within the 90 minutes before penicillin application and analyzed as reported above for seizures. Results are presented in **Supplementary material, Fig. S3**.

Declarations

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CONTRIBUTIONS

N.G., F.S.T., and J.C. conceived the original idea and designed the model. L.F. and F.S. carried out the experiments. L.F. collected the data, performed the analytic calculations and interpretation, with the support of A.N., and wrote the manuscript directly supervised by A.N. and N.G. All authors discussed the results, commented on the manuscript, and approved the final version to be published.

CONFLICTS OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

SUPPLEMENTARY MATERIAL FILE

Supplementary material file contains figures S1, S2, S3 and S4 and Table S1, S2 and S3.

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Figures

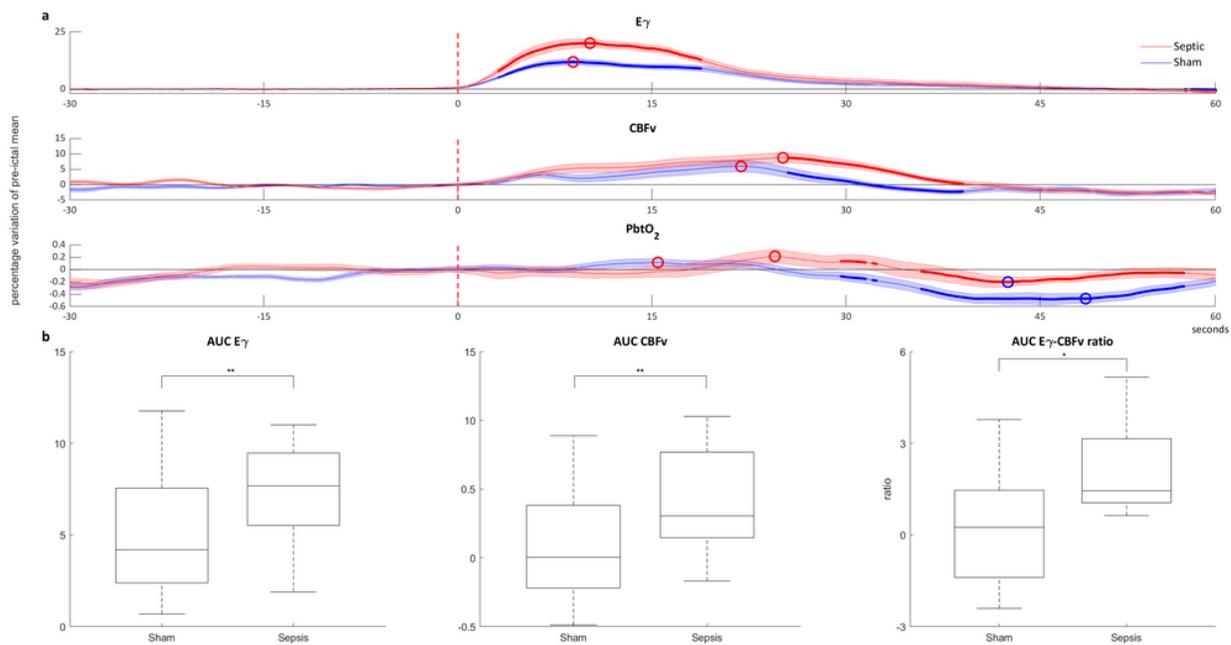


Figure 1

Local effects of seizures in the sham and sepsis groups (panel A) and comparison between groups of the area under the curve of the E γ and of the CBFv variations and their ratio (panel B).

Abbreviations: E γ : EEG gamma power envelope, CBFv: cerebral blood flow velocity, PbtO $_2$: partial tissue oxygen pressure, AUC: area under the curve, *: P < 0.01, **: P < 0.001. . Panel A: in blue, mean \pm SD seizure-induced value variation in time in sham group; in red, mean \pm SD seizure-induced value variation in time in sepsis group. Mean and SD are calculated using bootstrapping with replacement and 100 iterations. Circles correspond to the peaks of a parameter variation (red circles for the positive and blue for

the negative ones). Time 0 corresponds to the seizure onset (vertical dashed red line); curves were centered to zero at seizure onset. Differences between groups, identified by two-sample t -tests, are bolded and calculated on the raw data without bootstrapping. The increase in both CBFv and PbtO₂ is delayed in the sepsis group (see text for discussion). Parameters are expressed in percentage of variation from the mean of the last 15 pre-ictal seconds except for Ey, where baseline values were simply set to zero. Panel B: boxplots of the AUC of the Ey and of the CBFv variations and their ratio. An increase in the Ey and the CBFv AUC was noted in the sepsis group ($P < 0.001$ for both).

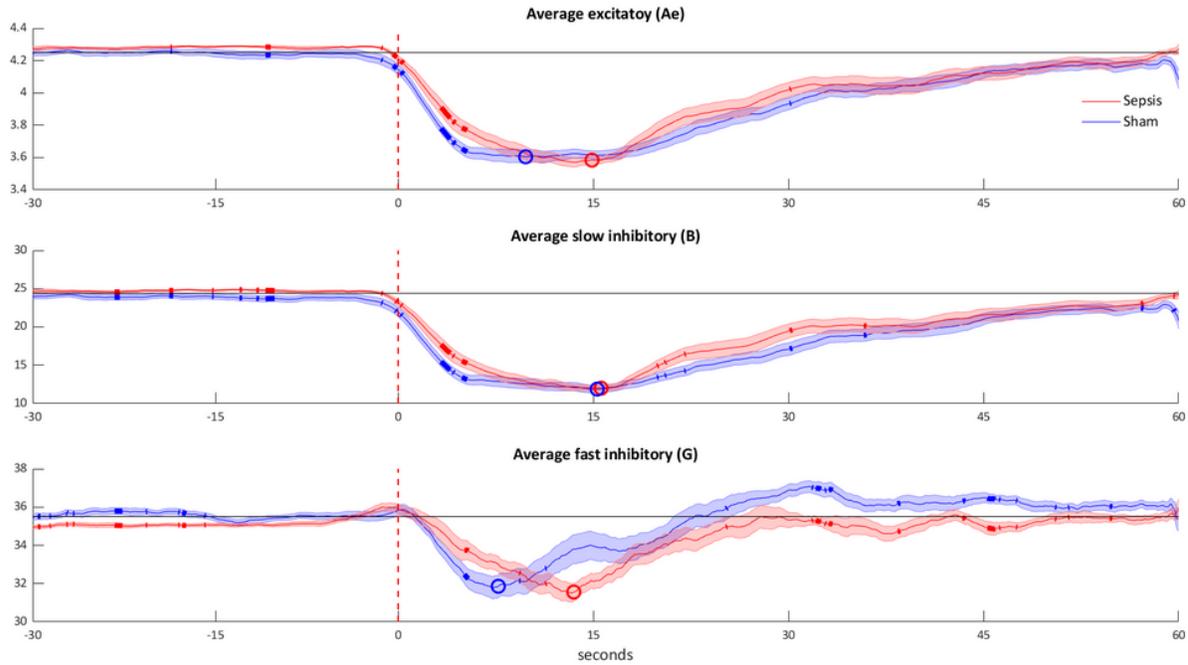


Figure 2

Temporal evolution of synaptic gain.

In blue, mean \pm SD seizure-induced value variation in time in sham group; in red, mean \pm SD seizure-induced value variation in time in sepsis group. Mean and SD are calculated using bootstrapping with replacement and 100 iterations. Circles correspond to the peaks of a parameter variation (blue circles for the sham and red for the sepsis group). Time 0 corresponds to the seizure onset (vertical dashed red line). Differences between groups, identified by two-sample t -tests, are bolded and calculated on the raw data without bootstrapping. For all the parameters, the descending part of the seizure-induced variation curve was steeper in the sham group. The dip was delayed in the sepsis group and particularly for the fast inhibitory synaptic gain variation that reached the dip with a lag of about 4.3 sec (IQR [-6.4-15] sec, $P = 0.06$, Table S3).

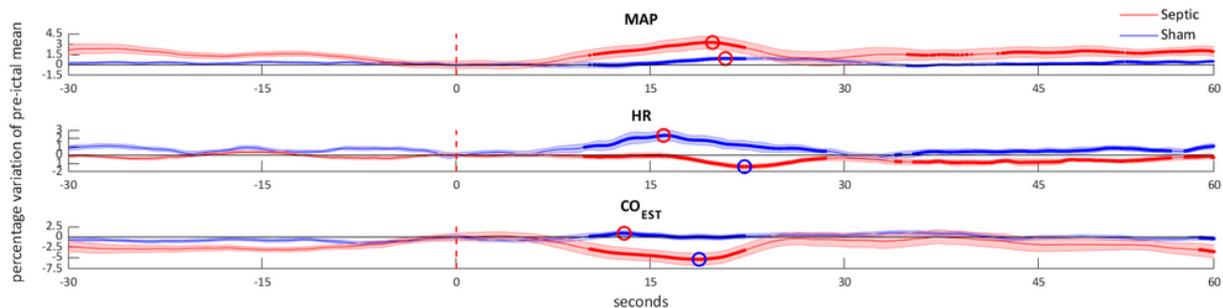


Figure 3

Systemic effects of seizures in the sham and sepsis groups.

Abbreviations: MAP: mean arterial pressure, HR: heart rate, CO_{EST}: cardiac output estimation. See Figure 1 for methodological explanations. The ictal MAP variation in the sepsis group is greater, whereas HR and CO_{EST} response is inverted in comparison to the sham group (see text for discussion).

Supplementary Files

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