# NEONATAL SEPSIS ALTERS THE EXCITABILITY OF REGULAR SPIKING CELLS IN THE NUCLEUS OF THE SOLITARY TRACT IN RATS

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## Abstract

**Objective:** Sepsis is a leading cause of mortality and morbidity in infants. Although the measures of autonomic dysfunction (e.g. reduced heart rate variability) predict mortality in sepsis, the mechanism of sepsis-induced autonomic dysfunction has remained elusive. The nucleus of the solitary tract hjh(NTS) is a vital structure for the integrated autonomic response to physiological challenges. In the present study we hypothesized that sepsis alters the excitability of NTS neurons in a rat model of neonatal sepsis (14-day old rats).

**Methods and results:** Sepsis was induced by intraperitoneal injection of cecal slurry (CS) in rat neonates. The presence of autonomic dysfunction was confirmed by observing a significant reduction in both short-term and long-term heart rate variably following CS injection. We investigated the effect of polymicrobial sepsis on the electrophysiological properties of the medial NTS neurons using a whole cell patch clamp recording. Our results showed that the resting membrane potential in regular spiking neurons was significantly less polarized in the septic group (-37.6 ± 1.76 mv) when compared with the control group (-54.7 ± 1.73 mv, $P<0.001$). The number of spontaneous action potentials in the septic group, was also significantly higher than the control group ($P<0.05$). In addition, the frequency and amplitude of the spontaneous excitatory post synaptic potentials (EPSPs) was significantly higher in neurons recorded in the septic group ($P<0.001$). Interestingly, regular spiking cells in the CS group exhibited a rebound action potential following hyperpolarization. Injection of depolarizing currents was associated with lower first spike latency and changes in rise slope of action potential ($P<0.001$).

**Conclusions:** We showed that polymicrobial sepsis increases the excitability of regular spiking cells in the medial NTS. These alterations can potentially affect neural coding and thus may contribute to an abnormal homeostatic or allostatic physiological response to sepsis and systemic inflammation.

# INTRODUCTION

Sepsis is recognized as a global health priority by the World Health Organization (1). This systemic illness is associated with progressive multiple organ dysfunction and high mortality in susceptible individuals, such as neonates (2–4). Reduced heart rate variability (HRV) is a hallmark of sepsis and markedly predicts mortality (5–7). There is evidence to suggest that the decreased HRV is caused by a partial uncoupling of the cardiovascular autonomic regulatory centers during systemic inflammation (8,9). Autonomic dysfunction in sepsis is not limited to the cardiovascular system as other visceral functions (e.g. such as in the hepatic and gastrointestinal tract) are also severely affected (10,11). Although the measures of autonomic dysfunction predict mortality in sepsis, the mechanism of sepsis-induced autonomic dysfunction has remained elusive.

The nucleus of the solitary tract (NTS) is a crucial structure involved in maintaining homeostasis of the autonomic system and its destruction is lethal (12,13). Amorim et al. recently demonstrated that acute endotoxin administration in rats is associated with a higher number of activated microglia and interleukin-1β levels in the NTS (14). Despite the crucial role of the NTS in the integrated responses to physiological and pathological challenges, it is not known how sepsis affects neural function in the NTS. In the present study we hypothesized that sepsis alters the excitability of NTS neurons and investigated the effect of polymicrobial sepsis on the electrophysiological properties of the regular spiking cells in NTS neurons. We chose the medial NTS for the electrophysiological recording as previous studies has indicated c-Fos staining is more prominent in the medial part of the NTS in animal models of systemic inflammation (15). Additionally, this region contains large neurons that send projections to the vagal dorsal motor nucleus and other autonomic centers in the brain stem (16). Kawai and Senba characterized the neuronal processes of NTS in rats and observed four types of projection neurons based on their response to depolarizing currents: (a) *regular spiking neurons* that fire multiple action potentials during current injection. (b) *single spiking neurons* that fire only one action potential during injection, (c) *late onset spiking neurons* exhibit a notch in the initial part of stimulation which is followed by delayed evoked action potentials. (d) *phasic spiking neurons* that exhibit phasic spikes during early phase of stimulation (16). We observed that the regular spiking cells are the most abundant cells in mNTS in rat neonates and thus investigated the effect of sepsis on the electrophysiological characteristics of these neurons. This report summarizes our findings in a rat model of neonatal polymicrobial sepsis.

# MATERIALS AND METHODS

### *Animals and ethics*

14-day old (p14) Sprague–Dawley male rats (body weight 21.33 ± 0.45 g) were used in this study. All animal procedures were approved by the Ethics Committee of Tarbiat Modares University and were in accordance with recommendations established by NIH guidelines (publication no. 85-23). We chose p14 rat pups in accordance to previous comparative studies between human and rats (17,18). Romijn et al., determined that the cerebral cortex of a new-born human is developmentally most comparable to that of a p12 to p13 rat pup (17). Other investigators also suggested that the first 12 days of life in rats are probably most comparable to the late gestational period of humans (18). Great care was taken to minimize the number of animals and their suffering. The number of required samples for our study were estimated based on Power calculation. In brief, with the assumption of 10 mv increase in resting membrane potential and 20% variability, we require 10 samples in each group to achieve 90% power with 5% error type I. Based on this, at least 10 samples were used in each experimental group.

### *Experimental model of polymicrobial sepsis*

Intraperitoneal injection of rat cecal slurry (CS) is a classic model for induction of peritonitis and polymicrobial sepsis in rodents (1,19,20). CS has been shown to induce a systemic inflammatory response syndrome and lead to colonization of bacteria in the blood, lungs and spleen in neonatal rodents (21). The CS was prepared using the cecal contents of a 12-week old healthy Sprague-Dawley rat as described (22) (100 mg dissolved in 1 ml saline solution) and was injected intraperitoneally (13 µl/g rat).

To confirm that this experimental model impairs autonomic control, linear and non-linear indices of HRV was calculated. An electrocardiogram (ECG) was recorded in alert rats in a custom-made Faraday's cage. Two patch electrodes (BlueSensor 2300, Ambu, UK) were gently placed at the left leg and right arm. Electrodes were then attached to a bio-amplifier and a digital data acquisition system with a sampling frequency of 10 kHz (Powerlab, ADInstrument, Australia). Cotton was then placed around the neonates to make them feel comfortable and body temperature was monitored with a noncontact infrared thermometer (Acumed HB 500, Switzerland). Rats were also kept on a heating pad (37 $^{o}$C) to prevent hypothermia during the ECG recording. 60 min after the baseline recording,

sterile saline or CS solutions were injected intraperitoneally. Respiratory rate was measured using the ECG-Derived Respiration algorithm (Kubios software).

***HRV analysis***

The R peaks were detected, and the R-R interval series was generated using a computer program developed in MATLAB. The R-R interval time-series was visually inspected, and the ECG recording was divided into 15 min artefact-free segments. The following indices were measured using a software developed in MATLAB:

*SDNN:* The standard deviation of the R-R intervals was calculated by the square root of the average of the squared individual differences. SDNN provides a measure of total HRV.

*SD1 and SD2:* The Poincaré plot graphically represents the correlation between consecutive R-R intervals. We used the Poincaré plot to calculate short-term and long-term variability. *SD1:* The standard deviation of the points perpendicular to the line of identity describing short-term variability, which is mainly related to the effects of respiration on the vagal drive (23). *SD2:* The standard deviation along the line of identity in the Poincare' plot, which describes the long-term HRV. Many physiological factors including thermoregulation and the baroreflex loop contribute to long-term HRV (24).

*Fractal-like scaling exponent:* Cardiac cycles exhibit fractal-like scaling behavior. Detrended fluctuation analysis (DFA) is a classic method to quantify fractal-like correlation properties in physiological time-series (25). In this method, a linear relationship between the log (fluctuations) and log(scale) indicates the presence of a fractal-like time series. The slope of this line (α) is the fractal-like exponent which can be separately calculated for short windows (scale ≤ 16) and long windows (scale > 16).

***Slice preparation***

Three hours after the CS or saline injection, neonate rats were anaesthetized with ethoxyethane and rapidly decapitated. The brainstem and cerebellum were quickly dissected from the whole brain and then gently immersed in ice cold (1– 4°C) cutting artificial cerebrospinal fluid (aCSF) for further trimming. Cutting aCSF composition (in mM) was as follows: 2.5 KCl, 0.5 $CaCl_2$, 2 $MgSO_4$, 1 $NaH_2PO_4$, 26.2 $NaHCO_3$, 217 sucrose, 1 L- ascorbic acid and 11 D-glucose (26). The trimmed brain block was glued on a cutting platform and then coronally sectioned by a vibratome (VT1200S, Leica,

USA). Two coronal slices of 300 µm thickness were obtained. Slices were then transferred to a standard aCSF under continuous bubbling by 95% $O_2$–5% $CO_2$. Standard aCSF contained (in mM); 132 NaCl, 2.5 KCl, 1 $NaH_2PO_4$, 25 $NaHCO_3$, 10 D- Glucose, 2 $CaCl_2$, 2 $MgSO_4$ and 1 L- ascorbic acid (27,28). The osmolarity and pH of cutting and standard aCSF were adjusted to 290-300 mOsm and 7.2-7.3 respectively (28).

***Whole cell patch clamp recording***

Slices were carefully transferred to a recording chamber containing standard aCSF with continuous bubbling by 95% $O_2$–5% $CO_2$. This solution perfused the slices at a flow rate of 1 to 2 ml/min. Slices were visualized by a fixed-stage upright microscope (Axioskop 2 FS MOT; Carl Zeiss, Gottingen, Germany) to find the medial NTS (mNTS), which is restricted between the area postrema, dorsal motor nucleus, central channel and solitary tract. mNTS neurons were identified by an IR-CCD camera (IR-1000, MTI, USA) with a 40x water immersion objective lens. Healthy neurons were selected and whole cell patch clamp procedure started under current clamp mode. Borosilicate glass pipettes (1.5 mm OD, 0.86 mm I.D) were pulled by a microelectrode puller (P-97, Sutter Instrument, USA). Pipettes were filled with intracellular solution containing (in mM); 115 K-gluconate, 10 HEPES, 11 EGTA, 2 $MgCl_2$, 10 NaCl, 2 MgATP and 0.25 $Na_2GTP$ (27). Tip pipettes resistance was (3–10 MΩ). The Data was collected under low-pass filtering at 10 kHz and acquired at 10 kHz with a Multiclamp 700B amplifier equipped with a Digidata 1440 A/D converter (Molecular Devices, Sunnyvale, CA, USA). The resting membrane potential was checked immediately after membrane rupture. Recording protocols were applied at least 5 minutes after cell membrane potential stabilization subsequent to membrane rupture. The spontaneous excitatory postsynaptic potentials (EPSP) were recorded during spontaneous activity of neurons (5 min) in the current clamp mode. Spontaneous EPSPs amplitude were measured as the voltage difference between the baseline to the peak of EPSPs as described by Cochran (29) using Clampfit software (version 10.4). Following five minutes recording of neural spontaneous activity, hyperpolarizing (-100 to -20 pA, 20 pA increments, 500 ms duration) and then depolarizing currents (20–180 pA, 20 pA increments, 500 ms duration) were applied as shown in Fig 4A (Vincent and Tell., 1997). All electrophysiological recordings were carried out at room temperature (23-25°C).

Only healthy neurons were recorded and considered for analysis. Morphologically, a healthy neuron has a round or oval-shaped soma without blemishes. Once the giga seal was achieved, the suitability of neurons was further evaluated at the electrophysiological level by measuring fundamental electrical properties such as stable resting membrane potential and capacitance. In our study, we applied

hyperpolarizing to depolarizing currents as described above. We checked the access resistance for every patched cell, before and after applying protocol. Neurons with an access resistance less than 25 MΩ were enrolled to the study. To ensure we recorded from healthy neurons, we didn't consider neurons for analysis if they showed more than 20% variations in access resistance during the study. Overall, two experimental groups, including animals receiving saline or CS, underwent patch clamp recording experiments.

***Statistical analysis***

Data are shown as Mean ± SEM. Student's t-test or its nonparametric equivalent (Mann-Whitney test) were used to compare the two groups. The number of cells was compared using Fisher's exact test. A two-way ANOVA was used to determine the effect that two independent variables (e.g. treatment or intensity of stimulation) had on a dependent variable (e.g. neural response to stimulation). Sidak's post-hoc test was used as a multiple comparison test. $P<0.05$ was considered statistically significant.

## RESULTS

Intraperitoneal CS injection elicited a significant rise in heart rate ($F_{group}=152.7$, $P<0.0001$, Fig 1A) as well as respiratory rate (100 ± 8 versus 130 ± 7 breath/min two hours after saline or CS injection respectively, $P<0.05$). As expected total HRV (SDNN) decreased significantly after CS challenge ($F_{group}=86.7$, $P<0.001$, Fig 1B). Poincaré plot analysis indicated that this reduction in HRV is reflected in both short-term (SD1) and long-term (SD2) heart rate fluctuations ($F_{group}=83.1$, $P<0.001$ and $F_{group}=98.4$, $P<0.001$ respectively, Fig 1C and D). Detrended fluctuation analysis showed that long-term fractal-like scaling $\alpha_2$ exponent changed significantly following CS administration as shown in S1 Fig (supporting information).

In the next part of the experiment, we investigated the electrophysiological characteristics of the mNTS neuron. Initially four types of neurons were observed in the mNTS based on their response to the injection of depolarizing currents as described previously (16)(S2 Figure). The most abundant type of neurons was a regular spiking neuron in both the CS and saline treated rats (as shown in supporting information S1 Table). Regular spiking neurons fire more than one action potential during the first step of depolarizing current injections. In this report, we focus on the electrophysiological properties of regular spiking neurons in neonates given CS or saline. Overall 16 cells from the saline group (10 p14 rats) and 14 cells from CS group (10 p14 rats) were recorded and analyzed.

As shown in Fig 2A, the resting membrane potential was significantly less polarized in the CS group (-37.6 ± 1.76 mv) when compared with the control group (-54.7 ± 1.73 mv, $P<0.001$). Both groups of cells, however, showed similar input resistance (557 ± 64 MΩ versus 654 ± 37 MΩ, $P=0.22$ in control and CS respectively). Most cells in the CS group exhibited spontaneous firing (Fig 3A). The number of spontaneous action potentials in the CS group (11 out of 14) was significantly higher than the control group (7 out of 16, $P<0.05$). We analyzed spontaneous EPSPs in cells from the saline and CS groups. As shown in Fig 2B and 2C, the CS injection was associated with a significant increase in both frequency and amplitude of spontaneous EPSPs ($P<0.001$, Mann-Whitney test).

Interestingly regular spiking cells in the CS group exhibited a rebound action potential following ~~the~~ hyperpolarizing currents (Fig 3B). None of the control cells showed any rebound firing. However, 21-42% of cells from the CS group exhibited rebound firing at different steps of the current injections (Table 1). Voltage sag (Vsag) was also measured during injection of hyperpolarizing currents (S3 Fig). As shown in Fig 4B, Vsag didn't changed in the CS group when compared with the control ($F_{group}=0.197$, $P=0.2$).

Regular spiking cells exhibited a regular repetitive spiking pattern throughout the depolarizing current injection in both experimental groups. Both the CS and the control groups behaved similarly and there was no significant difference in number of action potentials between these two groups (S4 Fig). The first spike latency in millisecond is depicted in Fig 4C. A two-way ANOVA indicated that the groups are significantly different in their first spike latency ($F_{group}=15.6$, $P<0.001$). We also looked at the amplitude, duration and slopes of first action potential during the injection of depolarizing currents. The amplitude of the action potentials in the CS group were lower ($P<0.001$, Fig 4E) while the duration of the action potentials were slightly higher in the CS group ($P<0.05$, Fig 4F) compared with the control. Likewise, both the rise, slope, (Fig 4D, $P<0.001$) and decay slope were significantly lower in the CS group when compared with the control.

## DISCUSSION

The present study was aimed at investigating the effect of neonatal sepsis on the electrophysiological characteristics of the regular spiking NTS neurons in rat neonates. Two main reasons motivated us to use a neonatal model of sepsis in our study. Firstly, the analysis of heart rate characteristics is currently used for early diagnosis of sepsis in neonatal intensive care units that are equipped with HeRO system (31). Secondly, whole cell patch clamp recording is more feasible and reliable in neonatal brain slices, in comparison with adult neurons. In the present study, we first demonstrate that polymicrobial sepsis induces autonomic dysfunction in P14 rats. Our results show that an injection of CS was associated with a significant elevation of heart rate, respiratory rate and a marked reduction of both short-term and long-term HRV. These results are in agreement with the effect of endotoxin on heart rate dynamics in rats as well as humans (8,9). We also showed that the fractal-like dynamics of heart rate fluctuation is altered in neonatal rats with systemic inflammation. The long-term fractal-like scaling exponent ($\alpha_2$) trended towards Brown noise-like fluctuations in septic rats, which indicates increased autocorrelation and memory of cardiac rhythm during systemic inflammation (24,32,33). Overall, our HRV study indicates that CS can alter heart rate dynamics in P14 rats similar to neonates with sepsis in human neonates.

In the second part of this study we looked at electrophysiological characteristics of the medial NTS (mNTS) neurons. We chose the mNTS, as previous studies reported that c-Fos expression is mostly elevated in mNTS in a rat model of systemic inflammation (15). Furthermore, this region contains medium to large cell bodies that project to the dorsal motor nucleus, nucleus ambiguous and ventrolateral medulla that are all involved in the integration of autonomic regulation (16,34,35).

Our results showed that the resting membrane potential in neurons recorded from the CS group is more depolarized than the controls. We also observed that a significant number of regular spiking mNTS neurons exhibited spontaneous firing in septic neonates. These results suggest that systemic inflammation in septic rats may alter resting ion currents (e.g. leaky potassium currents) and change excitability of these neurons in neonates with sepsis. This result is in agreement with a report by Chen et al. who looked at the electrophysiological characteristics of NTS neurons in a primate model of allergen-induced inflammation (36). Their results showed that extended allergen exposure could depolarize the resting membrane potential by 14% and markedly increased the excitability of these neurons. We also observed a significant increase in the amplitude and frequency of spontaneous EPSPs following polymicrobial sepsis. This finding suggests that the increased spontaneous firing

activity of regular spiking cell during sepsis might result from the rise in excitatory synaptic activity. Enhanced presynaptic glutamatergic neuronal activity is in line with the rise in spontaneous EPSP frequency. There is evidence to demonstrate that systemic inflammation is associated with neuroinflammation in the NTS (14). Neuroinflammation can potentially amplify the action of glutamatergic presynaptic terminals in NTS through cytokines (37,38). Nonetheless, the depolarization of the resting membrane potential has important physiological consequences for excitable cells. For example, the post-inhibitory rebound cells described only after sepsis, may have been revealed because of their depolarized resting potential and through the inactivation of low threshold conductance. Likewise, the reduced spike amplitude and increase in duration may be merely related to the depolarized resting potential. We did not investigate the mechanism of changes in spontaneous EPSPs in septic rats, and further studies are required to give mechanistic insights to this phenomenon.

Interestingly, the number of rebound action potentials following injection of hyperpolarizing currents increased in regular mNTS neurons in septic animals, a phenomenon which was not observed in control groups in our experimental setting. Neurons in some areas of central nervous system (CNS) may exhibit rebound firing after receiving trains of synaptic inhibition (39). This mechanism has a physiological function in different parts of the CNS (40) but its role in the pathophysiology of sepsis has not been reported. Medial NTS neurons receives both inhibitory GABAergic and excitatory glutamatergic synapses (41) and the process of rebound action potential in neurons from septic rats might have an impact in response to these inputs. Within the NTS, GABAergic inhibition plays an important role in the baroreceptor signal processing (42). It is well documented that the baroreflex sensitivity and blood pressure regulation is impaired in sepsis (14,43,44), however, the mechanism of this important abnormality is not well understood. There is no effective treatment for severe sepsis and the role of the CNS in the pathophysiology of sepsis has been overlooked until recently (45). Rebound firing may cause a paradoxical response to inhibitory signals at the mNTS and may impair autonomic regulation of visceral function in sepsis. However, further experiments are required to fully explore this hypothesis.

We found out that voltage sag did not show a notable change when comparing the septic group with the control neurons. This information shows that the hyperpolarization activated current ($I_h$) might not contribute in the generation of rebound firing in the mNTS neuron recorded from septic rats. There is evidence which supports the role of calcium currents in the occurrence of rebound action

potential firing in rat neonates (30). We did not study the mechanism of rebound action potential in our study and future experiments may pave the way in understanding the contribution and mechanism of this phenomenon in sepsis.

The first spike latency as well as the rise slope of the first evoked action potentials were reduced significantly in the regular spiking neurons in the septic group, following injection of depolarizing currents. This indicates that the active electrophysiological properties of regular-spiking cells are affected by polymicrobial sepsis. Altered electrophysiological parameters in sepsis including the elevated spontaneous action potentials and decrease in first spike latency may be a direct result of the depolarized membrane potential. Nevertheless, since first spike latency is involved in neural coding (46), these alterations may contribute to the abnormal homeostatic or allostatic neural response to systemic inflammation.

The NTS is involved in the regulation of visceral function including cardiovascular, respiratory, gastrointestinal as well as the immune system (45,47). Although sepsis is associated with cardiovascular manifestations, its effects are not restricted to the cardiovascular system and it profoundly impairs gastrointestinal as well as immune function (48). We chose the mNTS in this study for the electrophysiological recording, as previous studies had indicated that c-Fos staining is more prominent in the medial part of the NTS in animal models of systemic inflammation (15). Additionally, this region contains medium to large regular neurons that send their projections to the dorsal motor nucleus and other autonomic centers in the brain stem (16). Based on previous reports by Paton and colleagues, it appears that there is no obvious topography in the NTS for neurons controlling distinct visceral functions (13). Labelling studies have shown that that neurons responding to distinct afferent modalities (gastrointestinal tract, arterial baroreceptors, and peripheral chemoreceptors) are very close neighbors (13). This suggests that the same sub-region of the NTS can control multiple functions. Thus, the location of a region within the NTS does not necessarily implicate its function. This characteristic of NTS neurons may be important for integration of the physiological response to a variety of physiological challenges. The main limitation of our study is the lack of a direct link between increased excitability of regular spiking cells and impaired visceral function in sepsis. Future studies are required to understand the mechanism of this increased excitability and its potential role in the autonomic dysfunction in neonatal sepsis. Our results, however, do provide evidence that the mNTS neurons exhibit different electrophysiological properties during polymicrobial sepsis. There are only handful reports that suggest possible

involvement of NTS in sepsis (14) and, to the best of our knowledge, none of these reports explored electrophysiological properties of these neurons during systemic inflammation. Nevertheless, our study does not explore the mechanism of these electrophysiological alterations. This is a major limitation of our study which requires further investigation.

The NTS is an important hub for the visceral regulatory network and its complete dysfunction is lethal (13). Many patients do not survive severe sepsis and the most recent clinical trials that have targeted peripheral mechanisms have failed to reduce mortality in sepsis. We showed that regular spiking cells in the mNTS become more excitable in neonatal sepsis. This increased excitability could play a role in the pathogenesis of sepsis. Thus, redirecting the focus of sepsis research from end-organ target (e.g. myocardium and vasculature) to central neural mechanisms may introduce new targets for therapy in neonates with sepsis.

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**Table 1.** Number and percentage of cells with rebound action potentials following injection of hyperpolarizing currents (from -100 pA to -20 pA). CS: Cecal slurry. Fisher's exact test was used for statistical analysis. Data are expressed as ratio ( $\frac{number\ of\ cells\ with\ rebound\ action\ potential}{total\ number\ of\ cells}$) (%).

| | **-100 pA** | **-80 pA** | **-60 pA** | **-40 pA** | **-20 pA** |
|---|---|---|---|---|---|
| **Saline** | $\frac{0}{16}$ (0%) | $\frac{0}{16}$ (0%) | $\frac{0}{16}$ (0%) | $\frac{0}{16}$ (0%) | $\frac{0}{16}$ (0%) |
| **CS** | $\frac{5}{14}$ (35%) | $\frac{6}{14}$ (42%) | $\frac{5}{14}$ (35%) | $\frac{5}{14}$ (35%) | $\frac{3}{14}$ (21%) |
| **P-value** | 0.011 | 0.004 | 0.011 | 0.011 | 0.20 |

# Figures

**Fig 1.** The effect of cecal slurry on heart rate variability: The effect of cecal slurry (CS) or sterile saline on heart rate and heart rate variability (HRV) indices in neonatal rats. SDNN: Standard deviation of 15 min RR intervals in millisecond (ms). SD1 and SD2 represent short-term and long-term cardiac rhythm variability based on Poincaré analysis of HRV analysis. *** P<0.001 (for the effect of treatment in two-way ANOVA). [a] P<0.05, [b] P<0.01, [c] P<0.001 (Sidak's post-hoc test).

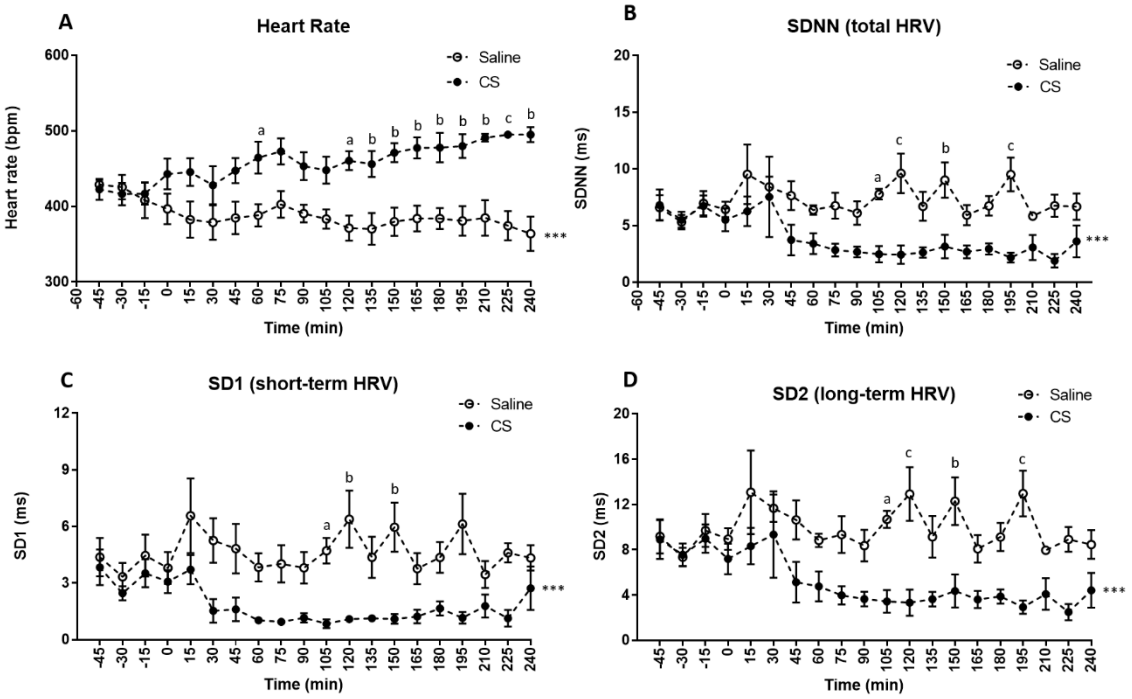


**Fig 2**. Electrophysiological characteristics of regular spiking mNTS neurons in cecal slurry (CS) or saline treated rats. A. Resting membrane potential, B. Frequency of spontaneous excitatory post synaptic potentials (EPSP), C. Amplitude of spontaneous EPSPs. *** P<0.001 (Student's t-test), ** P<0.001 (Mann-Whitney test).

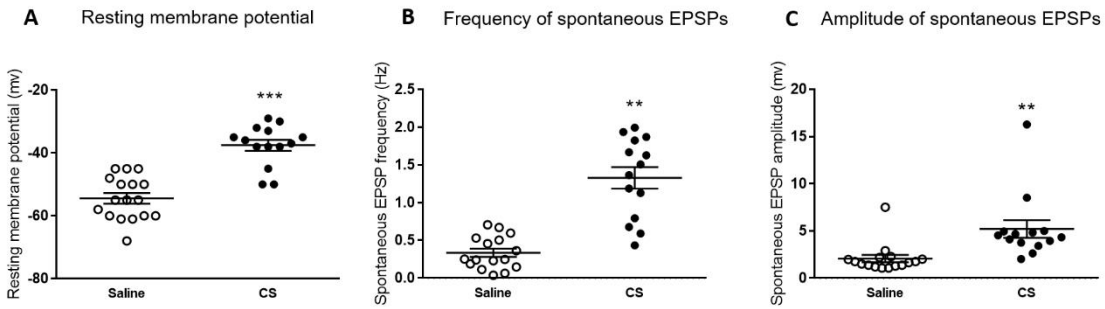


**Fig 3.** Typical sample recordings of spontaneous firing of neurons (A) and a rebound action potential after injection of hyperpolarizing currents (B) in the CS group.

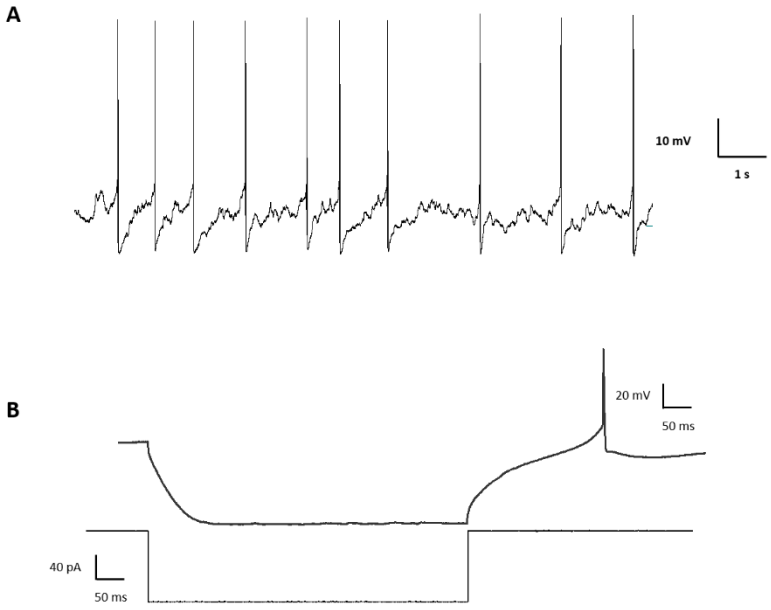
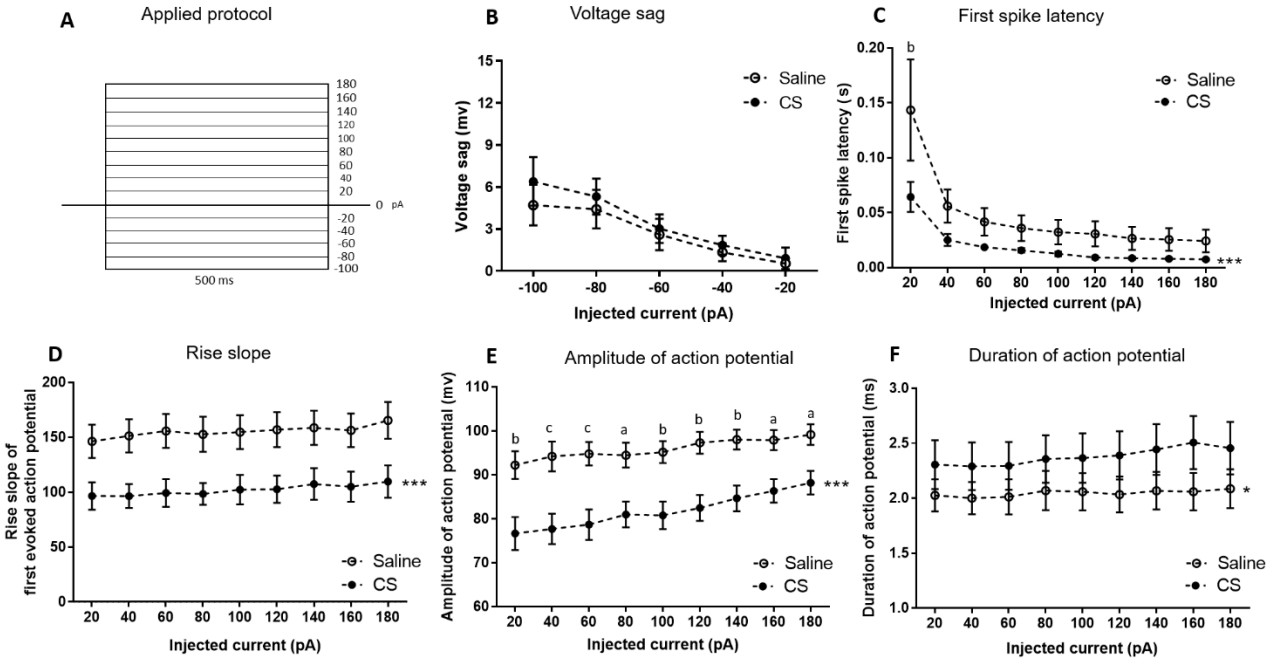
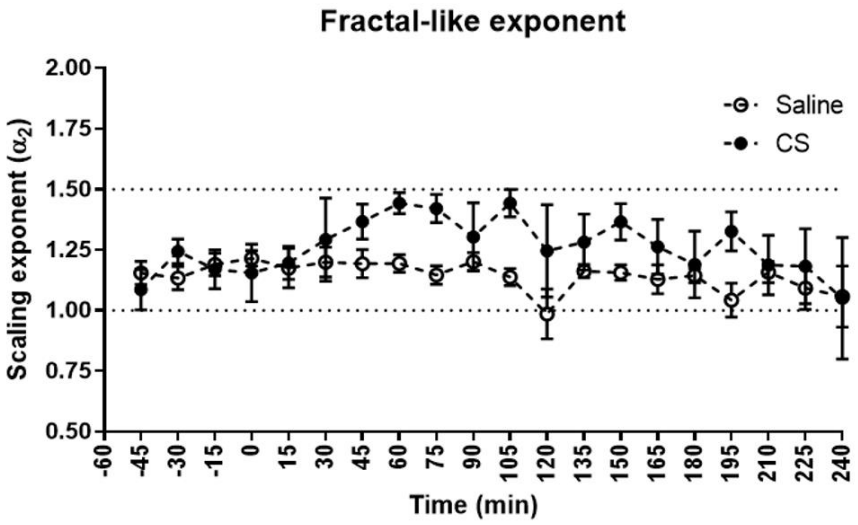


**Fig 4**. Electrophysiological characteristics of regular spiking mNTS neurons in cecal slurry (CS) or saline treated rats in response to hyperpolarizing or depolarizing current injections. A. Applied protocol, B. Voltage sag, C. First spike latency, D. Rising slope, E. Amplitude and F. Duration of action potential. * P<0.05, *** P<0.001 (for the effect of treatment in two-way ANOVA). [a] P<0.05, [b] P<0.01, [c] P<0.001 (Sidak's post-hoc test).

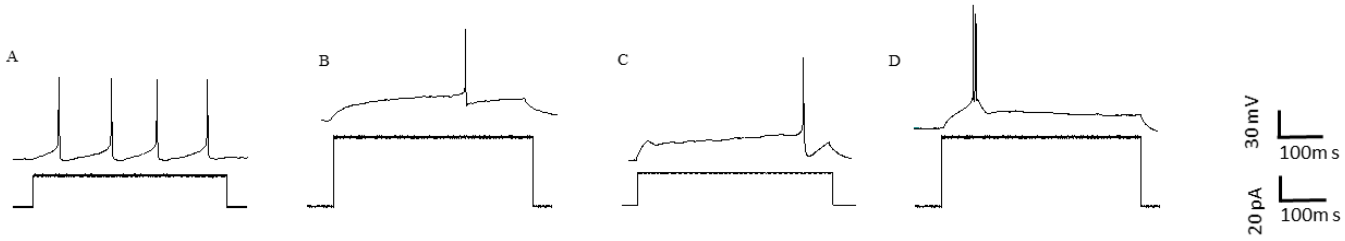


# Supporting information

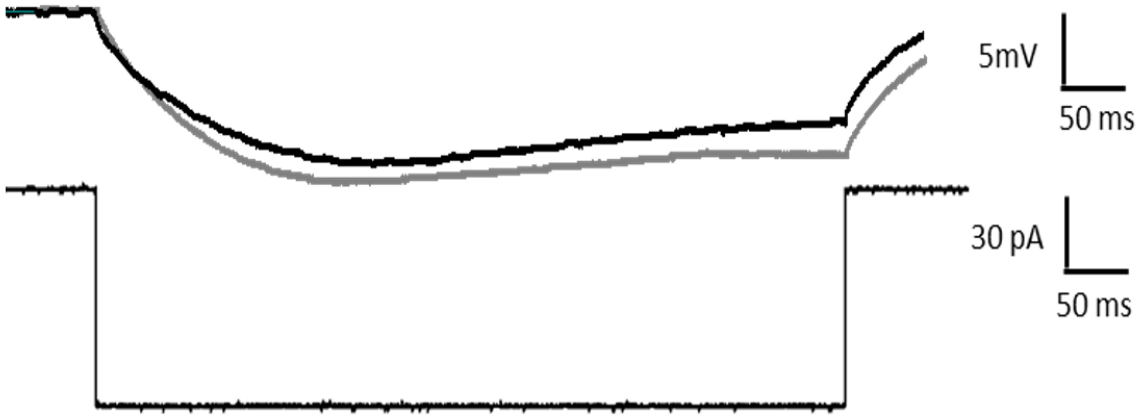
**S1 Fig. The effect of polymicrobial sepsis on fractal-like fluctuations of heart rate variability in neonatal rats**. Long-term fractal-like scaling ($\alpha_2$) exponent is close to 1 in saline group (compatible with a fractal-like 1/f dynamics) and polymicrobial sepsis changed this exponent towards 1.5 which is compatible with a Brown noise. Two-way ANOVA shows a significant difference for the effect of treatment ($F_{group}$=18.8, P<0.001). CS: Cecal slurry.



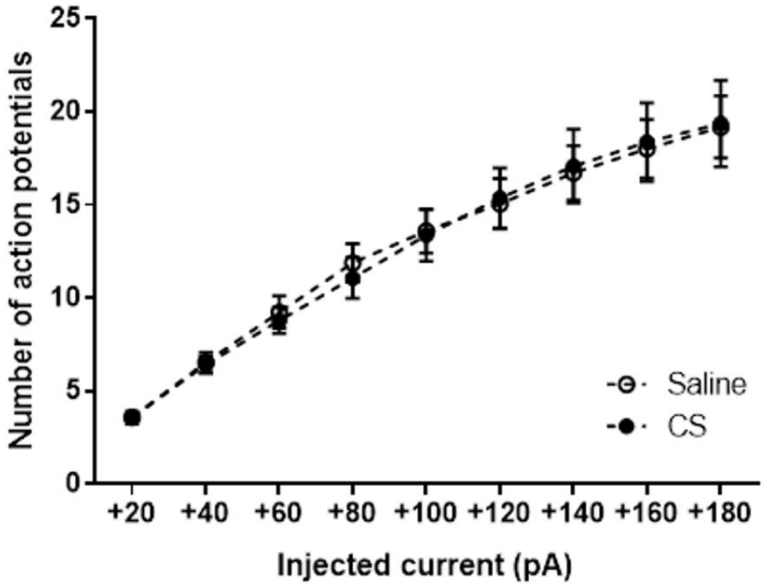
**S2 Fig. Four types of cells in medial NTS.** Medium to large medial NTS neurons (> 150 μm$^2$ in somal area) have been categorized into four types based on their response to a depolarizing current injection with 500 ms duration (Kawai and Senba, 1996): ***A. Regular spiking neurons*** fire more than one action potentials during current injection. Only this type of neurons is reported to fire rebound action potentials subsequent of hyperpolarizing currents. ***B. Single spiking neurons*** fire only one action potential during injection of the depolarizing current which may appear in the initial, middle or terminal phase of stimulation. ***C. Late onset spiking neurons*** exhibit a notch (shown by an arrow) in the initial part of stimulation which is followed by delayed evoked action potentials. ***D. Phasic spiking neurons*:** depolarization elicits phasic spikes during early phase of stimulation.



**S3 Fig**. **Voltage sag in regular spiking medial NTS neurons**. Voltage sag was measured in different hyperpolarizing currents as described in the material and methods section. This figure presents ploa sample for voltage sag analysed after injection of hyperpolarizing current (-60 pA) in saline (black line) and CS group (grey line).



**S4 Fig. Number of action potentials during depolarizing current injection in regular spiking cells.** There is no significant difference between the CS and Saline groups.



**S1 Table. Number of regular, single, late onset and phasic spiking cells in medial NTS recorded in saline and CS threated rats.**

| | Regular spiking | Single spiking | Late onset spiking | Phasic spiking |
|---|---|---|---|---|
| Saline | 16 | 5 | 5 | 3 |
| CS | 14 | 11 | 5 | 2 |