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Identification of vagus nerve stimulation parameters affecting rat hippocampal electrophysiology without temperature effects

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ABSTRACT

Background: Recent experiments in rats have demonstrated significant effects of VNS on hippocampal excitability but were partially attributed to hypothermia, induced by the applied VNS parameters.
Objective: To allow meaningful preclinical research on the mechanisms of VNS and translation of rodent results to clinical VNS trials, we aimed to identify non-hypothermia inducing VNS parameters that significantly affect hippocampal excitability.
Methods: VNS was administered in cycles of 30 s including either 0.1, 0.16, 0.25, 0.5, 1.5, 3 or 7 s of VNS ON time (biphasic pulses, 250μs/phase, 1 mA, 30 Hz) and the effect of different VNS ON times on brain temperature was evaluated. VNS paradigms with and without hypothermia were compared for their effects on hippocampal neurophysiology in freely moving rats.
Results: Using VNS parameters with an ON time/OFF time of up to 0.5 s/30 s did not cause hypothermia, while clear hypothermia was detected with ON times of 1.5, 3 and 7 s/30 s. Relative to SHAM VNS, the normothermic 0.5 s VNS condition significantly decreased hippocampal EEG power and changed dentate gyrus evoked potentials with an increased field excitatory postsynaptic potential slope and a decreased population spike amplitude.
Conclusion: VNS can be administered in freely moving rats without causing hypothermia, while profoundly affecting hippocampal neurophysiology suggestive of reduced excitability of hippocampal neurons despite increased synaptic transmission efficiency.

Introduction

Vagus nerve stimulation (VNS) is an approved therapy for drug-resistant epilepsy. Nevertheless, the exact mechanism of action remains to be discovered [1]. VNS has anticonvulsant properties in multiple seizure and epilepsy models, such as the pilocarpine model for limbic seizures [2], the cortical stimulation model for motor seizures [3], the pentylenetetrazole model for generalized seizures [4], and the kinding model for temporal lobe epilepsy [5] amongst others (extensively reviewed in Aalbers et al. [6]). Other studies have found VNS capable of increasing neural plasticity in healthy rats [7] as well as rats with nervous system damage [8–11]], a promising finding in the search of true neuromodulatory approaches in epilepsy and related network disorders. Pairing VNS with rehabilitative training facilitates the recovery in animal models of stroke [10,11], traumatic brain injury [9], and tinnitus [8]. Typically, preclinical studies have been used to justify clinical parameter settings such as stimulation frequency [12,13] and intensity [4,14–16].

Our group observed profound effects on hippocampal EEG and evoked field potentials in freely moving rats when clinical stimulation parameter settings were used. Specifically, a reduction in the frequency of the hippocampal theta rhythm and a reduction of EEG power was observed. The activation of hippocampal neurons in the dentate gyrus in response to activation of presynaptic axons of the perforant path, i.e. population spike (PS), was delayed but increased [15,16]. However, further research demonstrated that these neurophysiological effects were accompanied by a pronounced brain and body hypothermia strongly confounding the

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simultaneously recorded hippocampal excitability parameters [17]. This is problematic for two reasons. First, to date VNS-induced hypothermia has not been reported in humans. Second, hypothermia affects hippocampal EEG [18] and evoked potentials in a similar way as we had seen in response to VNS [19,20].

In a recent study we counteracted the hypothermia induction by applying radiant heat during VNS while hippocampal electrophysiology was monitored [21]. When hypothermia was negated during VNS, the reduction of hippocampal power was still evident. In addition, the activation of CA1 neurons in response to activation of presynaptic axons (i.e. CA1 PS evoked by electrical stimulation of afferent Schaffer collaterals) was now reduced.

To completely disentangle the hippocampal electrophysiological effects of VNS from the hypothermia, a VNS protocol without hypothermia induction is required. In this study, we therefore systematically investigated a set of VNS parameters while evaluating brain temperature. Next, we recorded dentate gyrus evoked potentials and EEG to determine whether the identified non-hypothermia inducing VNS parameters were able to affect hippocampal neurophysiology.

Materials and methods

Animals

Thirteen male Sprague Dawley rats (Envigo, the Netherlands, 350 g) were housed under controlled conditions of 20–22°C, 40–60% humidity with a constant light/dark cycle of 12 h per phase (lights on at 9 AM.). All procedures were approved by the local animal experimental committee (ECD 15/89, Ghent University Hospital, Ghent, Belgium) and were in accordance with the European directive 2010/63/EU.

Surgery

Surgery was performed as described in Larsen et al. [17]. Briefly, under isoflurane anesthesia (5% induction, 2% maintenance in medical O_2) a custom-made bipolar cuff electrode was wrapped around the left vagus nerve after dissecting the nerve from the carotid sheath. A quadripolar recording electrode consisting of four 70 μm polyimide coated stainless steel wires with 300 μm intertip distance was stereotactically implanted in the hilus of the dentate gyrus (-3.8 AP, +1.9 ML, -3 mm DV). A bipolar stimulation electrode (125 μm per wire, 900 μm intertip distance) was placed in the right dorsomedial perforant path (-8.1 AP, +3.9 ML, −2.5 mm DV). During electrode implantation evoked potentials were recorded to optimize dorsoventral location of the electrodes. A guide cannula (CMA/12, Aurora Borealis, The Netherlands) was implanted in the contralateral dentate gyrus (same coordinates as recording electrode) for thermocouple insertion. Post surgical analgesia was administered for up to two days after surgery (buprenorphine (0.03 mg/kg/day) and meloxicam (1 mg/kg/12 h)). Experimental sessions started 4 weeks after surgery.

Brain temperature and electrophysiological recordings

Rats were housed in dedicated cages for tethered, in vivo brain temperature and electrophysiology recordings. Brain temperature was recorded using a SE378 4-Channel Thermometer and a thermocouple (HYPO-33-1-T-G-60-SMPW-M, OMEGA) inserted via the guide cannula in the dentate gyrus. EEG signals were recorded via a head stage, carrying unity gain preamplifiers, and an electrical commutator connected to custom-built amplifiers (gain: x100; band-width: 0.159 Hz - 5.8 kHz). A stainless-steel epidural electrode, placed over the right frontal lobe, was used as ground and reference electrode. A data acquisition card (NI-USB-6259, National Instruments, USA, TX) was used to digitize the EEG (at 1 kHz) and EP (evoked potential) signals (at 20 kHz) with a 16-bit dynamic range and 3.05 μV resolution. Signals were stored on a computer for offline analysis. Stimulation pulses were delivered with custom-built constant-current stimulators.

In experiment 1, SHAM or active VNS were delivered in cycles of 30 s during 1 h after a 30 min baseline period. Four active VNS paradigms with different VNS ON durations per cycle (duty cycle) were compared to sham stimulation for their effect on brain temperature. The tested VNS ON durations included 0.5, 1.5, 3 or 7 s of biphasic pulse trains (1 mA, 250 μs/phase, 30 Hz). The 7-s condition was chosen as a positive control since we previously demonstrated clear hypothermic effects for this set of VNS paremeters [17,21]. Brain temperature was recorded every second during baseline and SHAM/VNS and this was continued for 1 h after SHAM/VNS.

Since all duty cycle conditions, apart from the 0.5 s condition, resulted in a clear brain hypothermia (see results), experiment 2 was conducted including lower VNS ON times (0.1, 0.16, 0.25, 0.5 and 7 s). In this experiment, SHAM/VNS were administered for 2 h to increase the chance to detect small temperature effects. During baseline and SHAM/VNS, EEG fragments of 10 s were recorded every 30 s. During active VNS, EEG recording started 4 s after the end of each VNS train. Every 180 s, two dentate gyrus EPs were recorded which were evoked 2 s after the VNS train during active VNS. The EPs were evoked by delivering two biphasic, square-wave electrical pulses with a duration of 100 μs/phase and an intensity that is 75% of the intensity evoking a maximal PS. The interstimulus interval was set at 20 ms which should result in a reduction of the second versus the first EP (paired-pulse inhibition).

Data preprocessing

Three parameters were extracted from the recorded hippocampal EPs (Fig. 1): 1) field excitatory postsynaptic potential (fEPSP) slope, by fitting a line from the fEPSP start until the PS onset using the least squares method; 2) PS amplitude, by measuring the vertical distance between the PS peak and the line between the positive fEPSP local peaks before and after the PS; 3) PS latency, by measuring the time between the stimulus onset and PS peak. Paired pulse inhibition was calculated as the ratio between the amplitude of the second PS and the first PS (PS2/PS1 ratio). EEG was filtered off-line with a first order Butterworth high pass filter (cut-off 1 Hz) and a notch filter at 50 Hz. To obtain local hippocampal EEG, signals from the electrode contacts yielding the smallest and largest evoked fEPSP were subtracted. Each 10-s EEG was split into 19 segments of 1 s, overlapping by 0.5 s and convoluted with a Blackmann-Harris window. Using Fast Fourier transform a power spectrum (1–100 Hz, 0.2 Hz resolution) was calculated for each 1-s segment. All 19 power spectra were averaged to obtain one power spectrum for each 10-s EEG. Theta peak frequency was determined as the frequency bin with the highest power within the theta frequency band (4–12 Hz). EEG segments were rejected due to artifacts if total power of that segment deviated more than 3 standard deviations from the total mean power.

Data analysis and statistics

EEG from all 13 rats was analyzed, while EP data was only used from 9 rats due to poor quality EPs in 4 rats. All data were processed using Matlab 2018a (The mathwork Inc., Natick, USA). Statistical analyses were performed using SPSS Statistics 25 (IBM Corporation, Armonk, New York, US) and R 3.3.2 (R Development Core Team).

For statistical comparison of VNS and SHAM-induced changes, hippocampal temperature, theta peak frequency and EP parameters

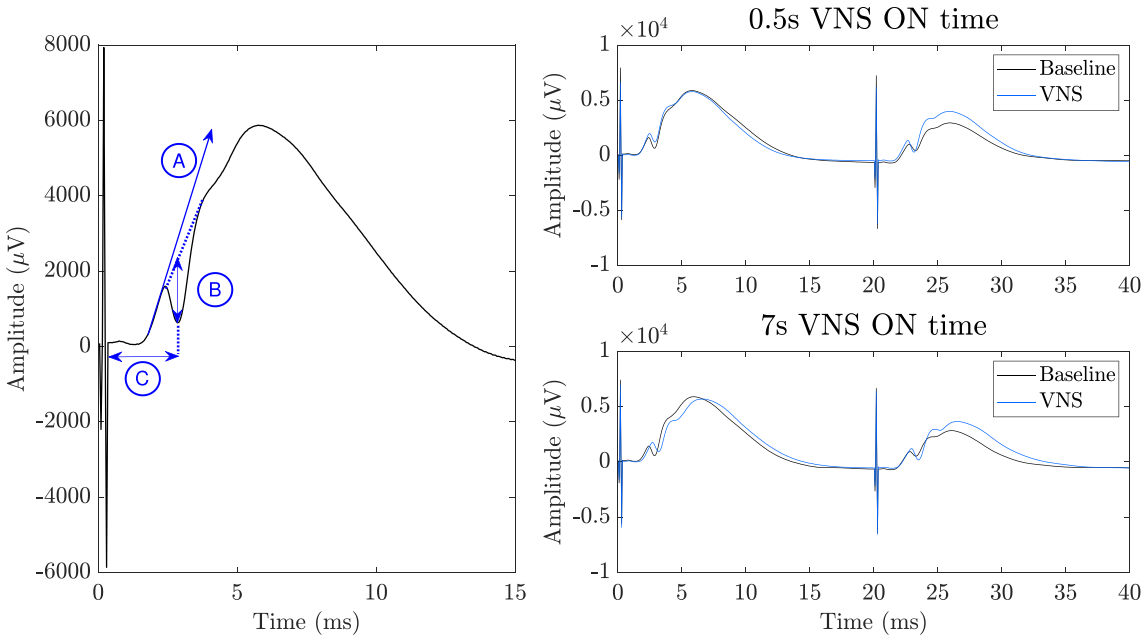

Fig. 1. *Left*; The three parameters extracted from an EP are A. the slope of the fEPSP, B. the amplitude of the population spike and C. the latency of the population spike. *Top right;* Representative sample of a dentate gyrus paired pulse evoked potential after perforant path stimulation during the 0.5 s VNS ON time condition. *Bottom right;* Representative sample of a dentate gyrus paired pulse evoked potential after perforant path stimulation during the 7 s VNS ON time condition. Baseline is depicted as a black line, while VNS is depicted as a blue line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

recorded during the last 30 min of VNS/SHAM (when the effects of VNS on temperature are most profound) were normalized to the respective averages of the 30 min baseline period. Only the 0.5 s VNS paradigm was selected for statistical comparison with SHAM and 7 s VNS because initial screening of brain temperature recordings (Fig. 1) showed that this was the most intense VNS paradigm that did not decrease brain temperature.

To allow meaningful comparison between the different biological measurements sampled at a different temporal resolution, temperature data (one value per second) and theta peak frequency (one value per 30 s) were down-sampled to match the sampling frequency of evoked potentials (one EP per 180 s). Therefore every 180^{th} sample for temperature and every 6^{th} sample for theta peak frequency was selected (after low-pass filtering with an 8^{th} order Chebyshev Type I infinite impulse response (IIR) filter [1/180 Hz (temperature) and 1/6 Hz (theta peak frequency) cut-off] to prevent aliasing).

A linear mixed effects model was fitted to the data with condition ('sham','0.5 s VNS' and '7 s VNS') as fixed effect and subject ID as random effect. The final model was $y_{ij} = (\beta_0 + u_{0j}) + (\beta_1 + u_{1j})X_{1ij} + e_{ij}$ with u_0 as random intercept of animals, β_0 as the global intercept, X_1 as condition and e as error term. Post-hoc multiple comparisons were performed between the different conditions with a Tukey correction using the 'multcomp'-package in R. The statistical significance was set at $p < 0.05$. All results represent mean values of the last 30 min of VNS, normalized by dividing with each animals' mean baseline and subsequently averaged over all animals±SEM.

To assess the effects of our different VNS conditions on the power of specific hippocampal EEG frequency bands a different normalization approach was adapted to account for the 1/f scaling property of EEG. EEG power spectra, measured during each VNS cycle of the last 30 min of SHAM/VNS (n = 60), were normalized to the average baseline EEG power spectrum per frequency bin using a dB conversion "$dB_{nf} = 10\log_{10}\left(\frac{Power_{nf}}{Baseline_f}\right)$" with n = duty cycle and f = frequency bin (0.2 Hz)). Normalized spectra were averaged and frequency bins were statistically compared between SHAM, 0.5 s and 7 s VNS using t-tests with a Bonferonni correction of 0.05/1500 to account for multiple comparison (500 frequency bins *3 groups).

Results

Effect of VNS on brain temperature

One hour of VNS with 1.5, 3 or 7 s VNS ON-time per 30 s cycle reduced brain temperature respectively with −1.7%±0.5%, −2.0% ±0.4% and −3.1% ±0.8% relative to baseline. These VNS-induced temperature changes were all highly significantly different from the SHAM condition (0.1% ±0.1%) ($p < 0.001$, Fig. 2). In the 0.5 s condition, effects on brain temperature were less pronounced (−0.6% ±0.3%) but still significantly different from SHAM ($p = 0.023$, Fig. 2).

A second experiment retested the 0.5 s VNS ON-time condition for effects on brain temperature and included lower duty cycles (0.1, 0.16 and 0.25 s ON-time), the 7 s ON-time condition as a positive control and a SHAM as a negative control. None of the duty cycles, except for the 7 s ON-time condition (−3.2% ±0.8%, $p < 0.001$), resulted in a significant brain hypothermia (Fig. 2). Next, the 0.5 s (VNS without hypothermia) and 7 s (VNS with hypothermia) conditions were selected for further analysis of their effects on hippocampal electrophysiology.

Effect of VNS on hippocampal evoked potentials

The 7 s VNS condition increased the PS latency with 8.3±1.8% which was significantly more than the 0.5 s condition (0.4±1.1%) and the SHAM condition (−0.4±0.4%). In the 0.5 s condition, VNS decreases PS amplitude with −12.8±5.9%, which was significantly different from the SHAM condition (1.0±8.9%, $p < 0.05$). In the 7 s condition, VNS increases PS amplitude (10.4±14.7%) which was

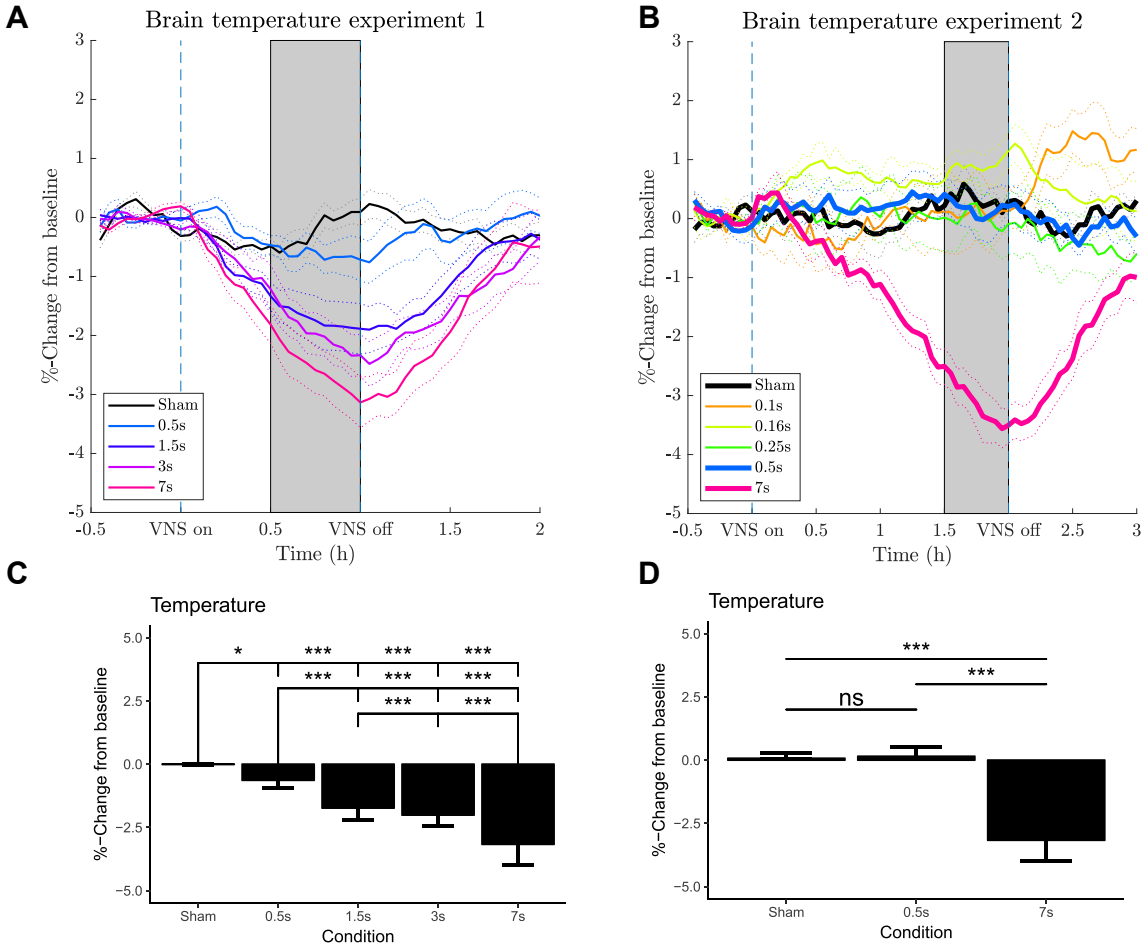

Fig. 2. A-D. Temperature changes due to VNS in the first and second titration experiment (±SEM depicted as dotted lines). In experiment 1, all VNS conditions resulted in temperature reduction, however the 0.5 s VNS condition did not share the same dynamics as the other conditions. Therefore experiment 2 was set up to increase the total stimulation time to 2 h which showed that the 0.5 s condition did not result in temperature changes significantly different from SHAM. Statistical comparison of temperature and electrophysiological parameters was performed on the last half hour of the VNS period (data in grey rectangle) for the SHAM, 0.5 s and 7 s conditions (thick lines).

significantly ($p < 0.001$, Fig. 3) different from the 0.5 s condition but not from SHAM ($p = 0.19$). Both in the 0.5 and 7 s VNS conditions fEPSP slope was increased with 10.6±3.8% and 12.3±3.5% respectively. Both increases were significantly different from the SHAM condition (0.8±2.0%, $p < 0.01$). Both in the 0.5 and 7 s conditions, PS2/PS1 ratio was increased respectively with 29.2±11.1% and 36.7±18.8% relative to baseline. Both increases were significantly different from the SHAM condition (−5.3%± 6.4%, $p < 0.001$).

Effect of VNS on hippocampal EEG

The changes of dentate gyrus EEG in the SHAM, 0.5 s VNS or 7 s VNS conditions relative to baseline are illustrated in Figs. 5 and 6. A reduction in theta peak frequency was seen (−10.4±1.8% relative to baseline) only in the 7 s condition, which was significantly different from both the SHAM and 0.5 s condition (−0.3±1.3% and −0.2±2.9% respectively, $p < 0.001$, Fig. 4). The 0.5 s and 7 s VNS conditions had different frequency-specific effects on the power spectrum. While the 7 s VNS condition reduced power of frequency bins ranging between 1-14.2 Hz and 35.8–100 Hz, 0.5 s VNS only reduced power in the 1–10 Hz range. No consistent effects of 0.5 s VNS in the higher frequency ranges could be demonstrated. Both for the 0.5 s as for the 7 s VNS condition no effects on power could be demonstrated in the 15–35 Hz range. In other words 0.5 s VNS mainly inhibits EEG in the delta-theta range, while 7 s VNS suppresses both delta, theta, and gamma frequencies.

Discussion

The primary objective of the current study was to identify VNS parameters that do not affect body temperature but still affect hippocampal excitability. These VNS parameters can be used in preclinical research to further investigate the mechanism of action of VNS and allow meaningful translation to the human situation. By reducing the amount of VNS ON time per 30 s from 7 to 0.5 s, hypothermic effects on VNS were prevented but profound effects of VNS on hippocampal electrophysiology were still observed. This 0.5 s normothermic VNS paradigm had significant effects both on the hippocampal EPs with an increased fEPSP slope, decreased PS amplitude and decreased paired-pulse inhibition and on the hippocampal EEG with a decreased power in the delta and theta frequency range.

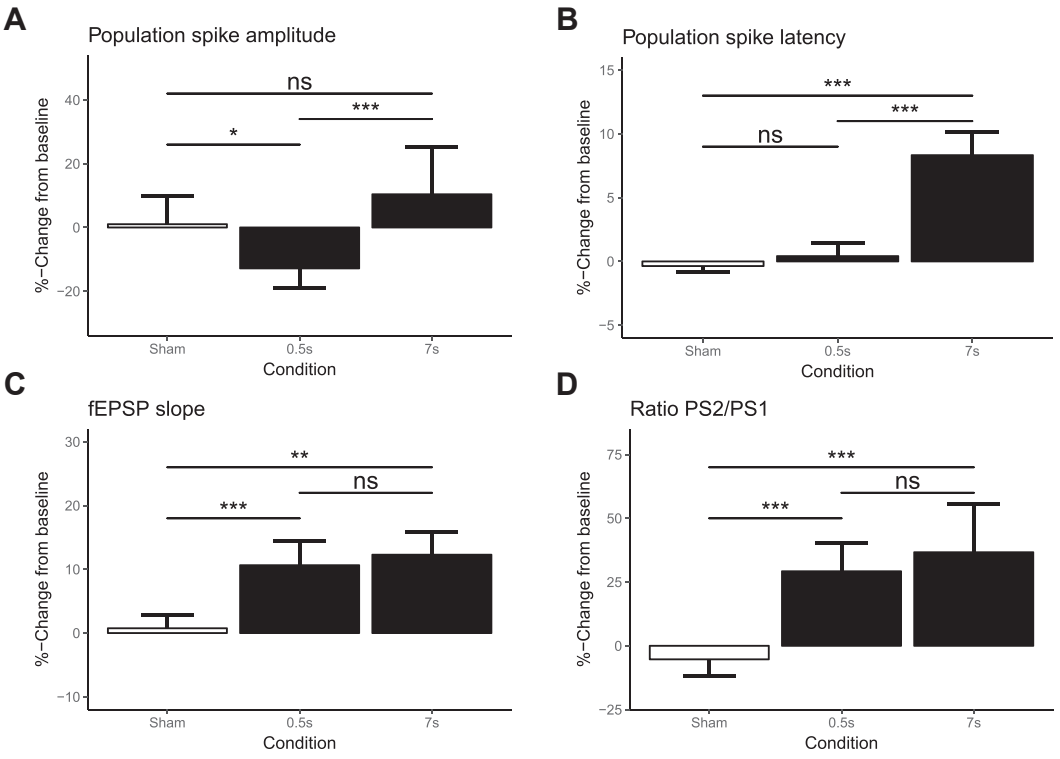

Fig. 3. A-D. PS amplitude, PS latency, fEPSP slope and paired pulse inhibition %-change from baseline for the different conditions, averaged over the last 30 min of the VNS period (+SEM). The significance after multiple comparison correction is depicted by horizontal lines above the bars. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Effects on temperature

It is still poorly understood how VNS modulates body temperature in rats. The most likely mechanism is through modulation of central thermoregulation (extensively reviewed in Morrison et al. [22]). The main afferent brain stem nucleus of the vagus nerve is the nucleus tractus solitarius (NTS). When body temperature increases, the NTS activates neurons in the dorsal part of the lateral parabrachial nucleus (LPB) which in turn activate GABAergic neurons of the preoptic area (POA) of the hypothalamus. The POA is the main regulator of body temperature through several thermoregulatory effectors [22]. Through one of their projections, POA GABAergic neurons inhibit cutaneous vasoconstriction (CVC) and brown adipose tissue (BAT) premotor neurons of the rostral raphe pallidus nucleus (rRPA), resulting in peripheral vasodilation and inhibition of BAT thermogenesis respectively [22]. Previous research in anesthetized rats indeed demonstrated that VNS (30 s train, 5–10 Hz, 1 ms pulses, 80–500 μA intensity) reduced BAT thermogenesis through inhibition of BAT premotor neurons [23]. Rather than a reduction of heat generation, our lab previously demonstrated that VNS results in a reduction of brain and body temperature by triggering an active heat release mechanism with vasodilation in non-hairy skin areas such as the tail [17]. The peripheral vasodilatation and active heat loss in response to VNS is likely to be mediated by inhibition of the CVC premotor neurons of the rRPA. The normothermic VNS paradigms, identified in this study, may fail to inhibit CVC premotor neurons or their inhibition is insufficient to result in a biologically relevant temperature reduction. Experiments that combine VNS with chemogenetic/optogenetic modulation of GABAergic rRPA neurons could further test this proposed mechanism of VNS-induced hypothermia.

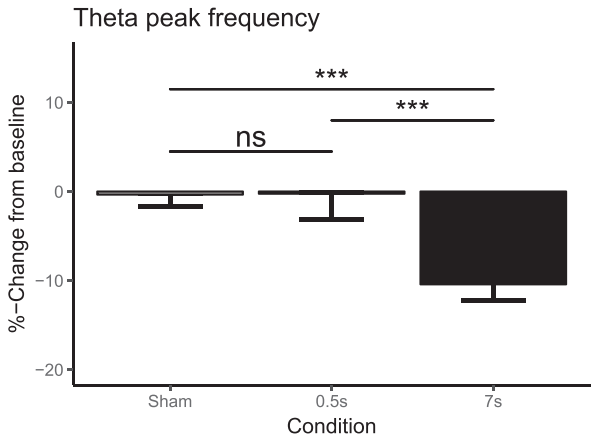

Fig. 4. Theta peak frequency %-change from baseline for the different conditions, averaged over the last 30 min of the VNS period (+SEM). The significance after multiple comparison correction is depicted by horizontal lines above the bars. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Effects on EP

The effects of VNS on dentate gyrus EPs are ambiguous. While an increased fEPSP slope supports increased efficacy of glutamatergic excitatory neurotransmission between perforant path and dentate gyrus granule cells, a decreased PS indicates reduced excitability of the dentate gyrus granule cells. The same effects were found by Ura and colleagues who studied VNS (biphasic pulses, 250 μs/phase, 1 mA, 30 Hz, 30s VNS ON every 5min for 60min) in anesthetized and temperature controlled (37.1 ±1°C) rats [24]. Shen and colleagues also demonstrated an increased fEPSP

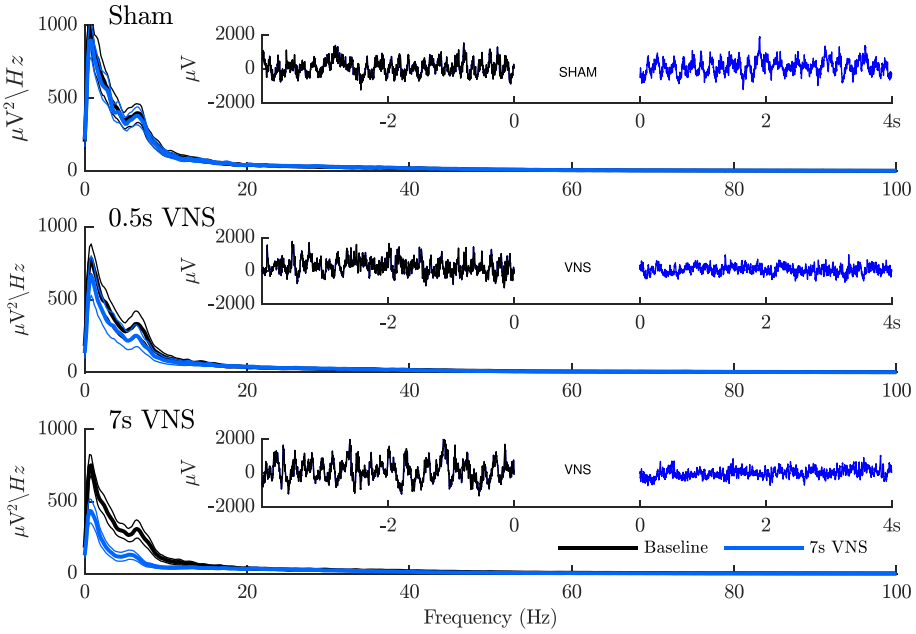

Fig. 5. Average power spectrum (1–100 Hz, ±SEM) of the 10 s EEG traces, recorded during the last half hour of the baseline (black trace) or SHAM/VNS period (blue trace). Insets show representative examples of 4 s hippocampal EEG during baseline (black) and SHAM/VNS (blue). Only 4 s are shown in order to illustrate the presence of theta rhythm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

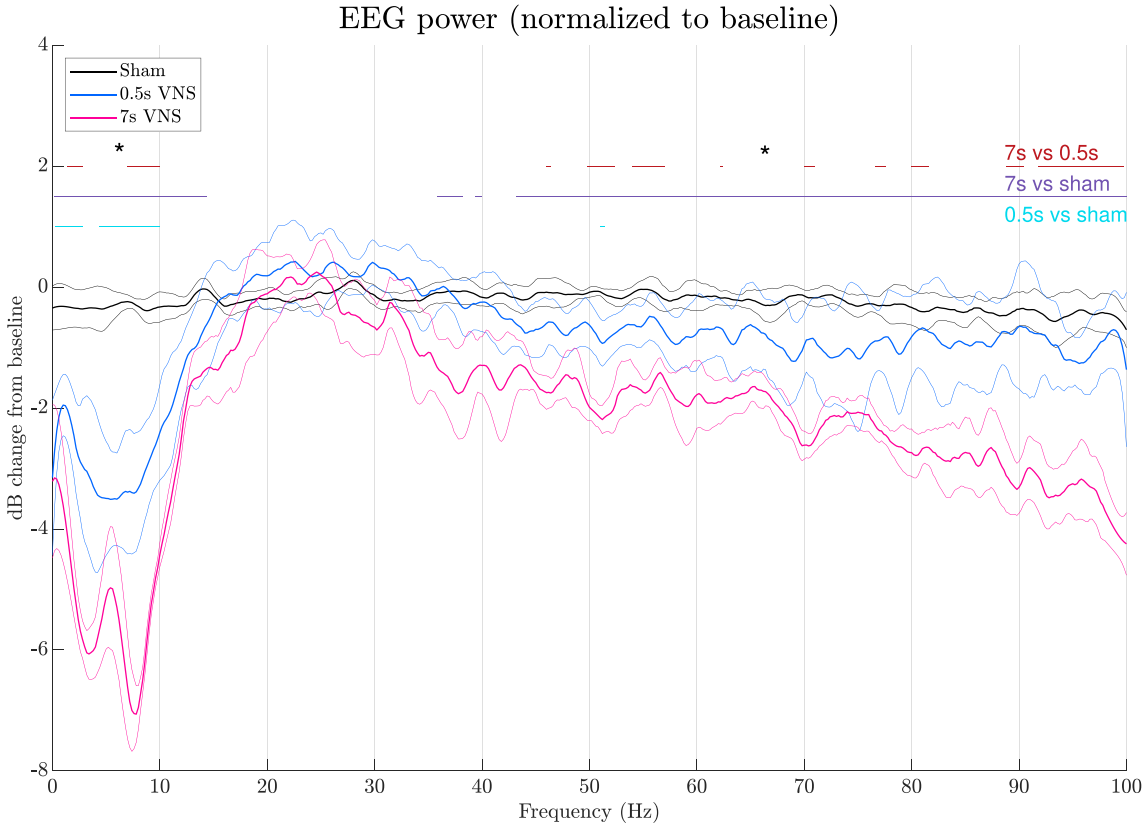

Fig. 6. EEG power (1–100 Hz, ±SEM) for the SHAM (black), 0.5 s VNS (blue), and 7 s VNS (purple) conditions after dB conversion relative to the 30-min baseline. Significance (*) of sample-specific t-tests are depicted by colour-coded lines above the lineplot. Note the significant difference between the 7 s VNS versus SHAM condition in both lower (delta-theta) and high (gamma) frequency range, while EEG power reduction in the normothermic 0.5 s VNS condition is significantly different from SHAM only in the lower (delta-theta) frequency range. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

slope in hippocampal CA3 region upon perforant path stimulation during VNS (biphasic pulses, 250 μs/phase, 1 mA, 20 Hz, 10 min VNS ON time). This VNS-induced effect on the hippocampal EP was blocked either by lesioning the Locus Coeruleus (LC), the sole source of norepinephrine (NE) in the hippocampus, or by pharmacologically blocking β-adrenergic receptors (AR) [25].

Several studies demonstrated activation of the LC-NE system in response to VNS [2,26–29]. In fact, the reduction of PS amplitude of the dentate gyrus (DG) EP could also be related to NE-dependent activation of β-adrenergic receptor (AR) signaling since local iontophoretic application of β-AR agonists/antagonists in dentate gyrus of anesthetized rats respectively decreased/increased PS amplitude of dentate gyrus EPs [30]. In addition, intrahippocampal administration of low concentrations of the non-selective β-AR agonist isoproterenol resulted in a transient suppression of DG PS amplitude. However, higher concentrations of the agonists resulted in opposite effects with increased PS amplitude [31].

It has been demonstrated that VNS is capable of driving LC neuronal depolarization [27,29]. Conversely, several studies showed that activation of the LC-NE system plays a crucial role in the seizure-suppressing effects of VNS [2,26]. In fact, selective activation [32] or lesioning [33] of the LC-NE system is sufficient to suppress/exacerbate epileptic seizures. Exactly how the seizure-suppressing effects of VNS-dependent LC-NE activation are mediated on the (sub)cellular/network levels is not well understood. NE has complex effects on the excitability of hippocampal networks since NE binds to different receptor subtypes (α_{1-2} and β_{1-3}-AR) located both pre- and post-synaptically on different cell types including neurons, astrocytes and microglia [34,35].

Other mechanisms, besides LC activation, are likely to be involved in the effects of VNS on hippocampal electrophysiology, given the widespread efferent projections of the Nucleus Tractus Solitarius (NTS) [36], the brainstem nucleus receiving most of the vagal afferent synapses [37]. For example, VNS also activates the habenula [38], a nucleus providing cholinergic input to the hippocampus [39]. Ura and colleagues suggested that activation of the M1 muscarinic receptor, which is widely expressed in the hippocampus [40], might be mediating the inhibitory effects of VNS on granule cell activation [24]. To elucidate the role of the different neuromodulators, hippocampal electrophysiology should be complemented with microdialysis, preferentially in combination with receptor specific antagonists. Alternatively, new cell-type specific neuromodulation techniques such as optogenetics [41] or chemogenetics [42] could target the different brainstem nuclei speculated or known to be involved in the MOA of VNS to further elucidate their role in VNS and/or modulation of hippocampal electrophysiology.

The VNS-induced reduction in PS amplitude may reflect (part of) its anticonvulsant mechanism of action. Both the classical AED, diazepam [43], as well as levetiracetam [44,45], a new generation AED, have been shown to decrease dentate field PS amplitude in anesthetized rats.

An increase in the PS2/PS1 ratio, indicating a decreased paired pulse inhibition, could reflect a reduction in the recruitment of $GABA_A$-receptor mediated inhibition [46]. This is not in line with a longstanding hypothesis which states that the anti-epileptic mechanism of action of VNS is based on enhancement of $GABA_A$-mediated inhibition [47]. In fact, the increased fEPSP slope also argues against an increase of GABAergic inhibition in response to VNS.

Effects on EEG

In line with previous studies, VNS reduced EEG spectral power [15–17]. This could reflect a decrease in excitability as EEG is predominantly an aggregate of synaptic potentials [48]. The decrease in EEG power was most pronounced in the delta and theta frequency ranges. This is in line with the initial findings of Zanchetti et al. who describe 'desynchronization' of the EEG in decerebrated cats during VNS [49]. The mechanism responsible for this effect likely lies upstream of the NTS, as electrical stimulation of the NTS at frequencies of 30 Hz and above similarly induces EEG desynchronization [50]. It has been speculated that the VNS-induced LC activation could be responsible for this 'desynchronizing' effect [51]. In awake, freely moving rats, electrical stimulation of the LC produces long-lasting inhibition of spontaneous activity of hippocampal neurons [52]. However, narrow band theta range (7–9 Hz) dentate gyrus EEG power of freely moving rats has been shown to increase rather than decrease after LC activation by local glutamate injection [53]. Cortical desynchronization may also be mediated by vagal afferent activation of other nuclei of the brainstem reticular formation [54], or by thalamic [55,56] activation, through the NTS [54].

The changes in excitability of dentate granular cells may be related to changes in the hippocampal EEG, especially in the theta frequency range. Action potentials in granular cells have been shown to be phase locked to oscillations of the local field potentials [57]. In vivo whole cell patch-clamp recordings of rat DG cells have demonstrated theta-oscillation coherent excitation [57]. Furthermore, Tsanov et al. have demonstrated that amplifying the power of endogenous theta oscillations, by using low frequency perforant path stimulation, increases dentate population spike amplitude and decreases fEPSP slope [58], while we have shown that VNS reduces theta power, decreases population spike amplitude and increases fEPSP slope.

In our study only healthy animals were used. In order to correlate the effects of VNS on hippocampal EPs to its anti-epileptic effects, replication in animal models for epilepsy is warranted since hippocampal EPs are altered in epileptic animals (increased PS amplitudes [59] and longer fEPSP duration [60]).

Conclusion

VNS can be performed in freely moving rats without causing hypothermia but still affecting hippocampal neurophysiology. In the absence of hypothermia, VNS reduces the excitability of granule cells and the power of hippocampal EEG, while it paradoxically increases efficiency of excitatory synaptic transmission and decreases inhibitory transmission supporting its potentially promising neuromodulatory effect. Future studies should investigate to which extent the observed hippocampal modulation correlates with the anti-epileptic effects of VNS.

Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Wouter Van Lysebettens: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Visualization. **Kristl Vonck:** Supervision, Writing - review & editing. **Lars Emil Larsen:** Software. **Latoya Stevens:** Resources. **Charlotte Bouckaert:** Resources. **Charlotte Germonpré:** Resources. **Mathieu Sprengers:** Writing - review & editing. **Evelien Carrette:** Project administration. **Jean Delbeke:** Writing - review & editing. **Wytse Jan Wadman:** Software. **Paul Boon:** Supervision. **Robrecht Raedt:** Conceptualization, Funding acquisition, Formal analysis, Writing - review & editing.

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