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High-Frequency Burst Vagal Nerve Simulation Therapy in a Natural Primate Model of Genetic Generalized Epilepsy

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Abstract

Purpose—Since the approval of Vagal Nerve Stimulation (VNS) Therapy for medically refractory focal epilepsies in 1997, it has been also reported to be effective for a wide range of generalized seizures types and epilepsy syndromes. Instead of conventional VNS Therapy delivered at 20–30 Hz signal frequencies, this study evaluates efficacy and tolerability of high-frequency burst VNS in a natural animal model for genetic generalized epilepsy (GGE), the epileptic baboon.

Methods—Two female baboons (B1 *Ph. Hamadryas* and B2 *Ph. Anubis* × *Cynocephalus*) were selected because of frequently witnessed generalized tonic-clonic seizures (GTCS) for VNS implantation. High-frequency burst VNS Therapy was initiated after a 4–5 week baseline; different VNS settings (0.25, 2 or 2.5 mA, 300 Hz, 4 vs 7 pulses, 0.5–2.5 sec interburst interval, and intermittent stimulation for 1–2 vs for 24 hours per day) were tested over the subsequent 19 weeks, which included a 4–6 week wash-out period. GTCS frequencies were quantified for each setting, while seizure duration and postictal recovery times were compared to baseline. Scalp EEG studies were performed at almost every setting, including intermittent light stimulation (ILS) to evaluate photosensitivity. Pre-ILS ictal and interictal discharge rates, as well as ILS responses were compared between trials. The Novel Object test was used to assess potential treatment effects on behavior.

Results—High-frequency burst VNS Therapy reduced GTCS frequencies at all treatment settings in both baboons, except when output currents were reduced (0.25 mA) or intermittent stimulation was restricted (to 1–2 hours/day). Seizure duration and postictal recovery times were unchanged. Scalp EEG studies did not demonstrate treatment-related decrease of ictal or interictal

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epileptic discharges or photosensitivity, but continuous treatment for 120–180 seconds during ILS appeared to reduce photoparoxysmal responses. High-frequency burst VNS Therapy was well-tolerated by both baboons, without cardiac or behavioral changes. Repetitive muscle contractions involving the neck and left shoulder girdle were observed intermittently, most commonly at 0.5 interburst intervals, but these were transient, resolving with a few cycles of stimulation and not noted in wakefulness.

Conclusions—This preclinical pilot study demonstrates efficacy and tolerability of high-frequency burst VNS Therapy in the baboon model of GGE. The muscle contractions may be due to aberrant propagation of the stimulus along the vagal nerve or to the ansa cervicalis, but can be reduced by minimal adjustment of current output or stimulus duration.

Keywords

High-frequency burst VNS Therapy; Generalized tonic-clonic seizures; Baboon; Idiopathic generalized epilepsy; Outcome

1. INTRODUCTION

Vagal Nerve Stimulation (VNS) Therapy provides an effective treatment for medically refractory focal epilepsies. Initially, the therapeutic effects of VNS were mediated by chronic intermittent stimulation as well as the ability to prevent or abort seizures by activating the device with a handheld magnet (Ben-Menachem, 2001; Morris and Mueller, 1999). The most recent model, the AspireSR® (Model 106), also provides stimulation in response to heart rate changes that may be associated with focal seizures (Boon et al., 2015). The original FDA approval for VNS Therapy hinged on the comparative efficacy between “high” (30 Hz frequency, pulse duration 500 microseconds, 5 minute intervals, magnet activation available) vs “low” (1 Hz, pulse duration 130 microseconds, 3 hour intervals, magnet activation not available) stimulation groups (Handforth et al, 1998). In the initial studies, a greater than 50% seizure reduction was experienced by 23–43% patients, with the responder rates increasing over the course of 3 years (Ben-Menachem, 2001; Morris and Mueller, 1999). In the meantime, Cyberonics (Houston, Texas, a fully owned subsidiary of LivaNova US Inc.) developed a new treatment paradigm utilizing high-frequency burst stimulation at 100–350 Hz, instead of the currently approved 20–30 Hz, in order to improve tolerability and efficacy. High-frequency burst stimulation refers to 4 to 7 pulses of high-frequency stimulation, each pulse lasting 250 microseconds with interburst intervals of 0.5 to 2.5 seconds. Initial tests of high-frequency burst stimulation in rodent and nonhuman primates models showed efficacy and tolerability (Alexander and McNamara, 2012; Ito and Craig, 2005). The rationale for adjusting the number of pulses per burst and varying the interburst intervals is to optimize the post-synaptic potentials of thalamic neurons. Paired-pulse stimulation of the vagus nerve potentiated post-synaptic potentials in the parafascicular nucleus of the thalamus compared to single pulse stimulation (Ito and Craig, 2005; Ito and Craig, 2008).

Over fifty years ago, nonhuman primates provided an important resource for the development and testing of antiepileptic medications and neurostimulation (Naquet and Meldrum, 1972; Hemmy et al., 1977; Killam, 1979; Lockard et al., 1990). One of the most

studied models, the baboon, exhibits natural, genetic generalized epilepsy (GGE) with photosensitivity (Naquet and Meldrum, 1972; Killam 1979). The modulation of photoconvulsive responses was utilized for testing medication effects in the epileptic baboon. Experimental nonhuman primate models, such as the alumina-gel model of focal epilepsy in the macaque, also played a significant role in the development of VNS Therapy (Lockard et al., 1990). The main advantages of nonhuman primate models include the neuroanatomic similarities to humans for better understanding of the electrophysiological effects and tolerability of VNS Therapy.

While there are several studies reporting efficacy of VNS in idiopathic or genetic generalized epilepsies, most of them are retrospective (Ng and Devinsky, 2004; Holmes et al., 2004; Kostow et al., 2007). This study, utilizing a well-documented model of GGE in the baboon, which closely corresponds to human idiopathic generalized epilepsies, represents an attempt to demonstrate efficacy and tolerability of high-frequency burst VNS Therapy over a five-month period. Primary treatment outcomes included change in generalized tonic-clonic seizures (GTCS) across the different treatment settings, measured as a weekly seizure average, while secondary outcomes included ictal or interictal changes on scalp EEG, including photosensitivity, as well as behavioral effects of VNS Therapy.

2. MATERIALS & METHODS

2.1. Animal Selection

Two baboons were selected on the basis of their baseline frequency of generalized tonic-clinic seizures (GTCS). One 16 year-old female baboon (B1; *Ph. Hamadryas*) had seizures mainly upon awakening, which increased during the course of her pregnancy and peripartum period. She was witnessed to have had 15 GTCS over a two-year period. The second 10 year-old female baboon (B2; *Ph. Anubis* × *Cynocephalus*) was noted to have GTCS in each of six sedations using ketamine within the preceding three years, and evidence of chronic periorbital scarring, which can be a marker for epilepsy (Szabó et al., 2014). Otherwise, both baboons were neurologically intact and otherwise healthy. The baboons underwent baseline scalp EEG studies and MRI scans of the brain (R21 N084198). Scalp EEG studies were repeated 4 times in B1 and 8 times in B2 (2 of which were excluded due to technical difficulties or alternate sedation), all confirming the diagnosis of GGE with or without photosensitivity. The brain MRI scan of B1 was normal, but B2 had an enlarged left occipital horn, or colpocephaly, which is also highly correlated with craniofacial trauma, and hence is likely to be the result of, and not the cause for, seizures (Szabó et al., 2016). All experiments complied with the ARRIVE guidelines (<http://www.nc3rs.org.uk>). These studies were approved by the Institutional Animal Care and Use Committee of the Texas Biomedical Research Institute. The baboons were treated in strict accordance with the United States Public Health Service's *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Research, 2011) and the *Animal Welfare Act* (Animal Welfare Act, 2008).

2.2. VNS Implantation

Both animals were implanted with a VNS Therapy System[®] (Cyberonics, Houston, TX), consisting of a pulse generator (Demipulse[™] Model 103) and a lead with helical electrodes (single-pin bipolar lead, 3.0 mm size). The research versions of Model 103 VNS devices are capable of delivering high-frequency burst stimulation (Figure 1). Under general anesthesia, the vagal nerve was dissected out of the vagosympathetic trunk (lies laterally to sympathetic portion) and the lead was coiled around it. The generators were placed interscapularly, both to keep the device out of reach and to avoid injury to the air sacs. During the surgery, the end tidal CO₂, inspiratory oxygen saturation and peripheral hemoglobin concentration were monitored continuously. After checking the impedance, high-frequency (300 Hz) burst VNS Therapy was delivered in the operating room with ECG monitoring at current outputs beginning at 0.25 mA, and gradually increased to 2.5 mA, with no observed alteration of heart rate. No heart rate changes were noted. Both baboons received buprenorphine 0.01 mg/kg for up to 48 hours postoperatively. The device was turned on in B1 at the time of surgery, while in B2 the device was only turned on 4 weeks after the surgery, again after titrating the current output to 2.5 mA while sedated with ketamine, in both cases closely monitoring the heart rate.

2.3. Study Design

The baboons were housed in single cages, which were placed side-by-side. Lights were turned on and off 12 hours at a time (usually 7 am and 7 pm), but often turned on briefly between midnight and 1 am for a few minutes, while veterinary technicians were checking on the animals. An Axis (Lund, Sweden) camera with infrared capabilities for night-time recordings, was set up with a frontal view to one or both cages from a three-meter distance. B1 was monitored for 24 weeks (including a five-week baseline, and a four-week pause), whereas B2 was monitored for 23 weeks (including a four-week baseline, and a six-week pause).

The overall strategy for adjusting VNS settings, was first to change interstimulus intervals, thereafter adjusting the number of pulses per burst as well as interburst intervals. Once VNS Therapy was initiated, there were slight deviations in their treatment protocols, some unintended, partly due to the programing interface. Signal frequency for burst stimulation remained at 300 Hz throughout the study. The following VNS settings were tested over 1–4 week periods (Table 1): stimulation intervals (1.8 vs 5 minutes), number of pulses (4 vs 7), and interburst intervals (IBI; 2.5, 1.5 or 0.5 secs) in order to optimize efficacy. Due to the diurnal pattern of seizures in B1 stimulation duration and times was adjusted (1 hour or 2 hours before awakening vs 24 hours), and in order to reduce aberrant peripheral nerve stimulation, so were current outputs (0.25 mA, 2 and 2.5 mA).

2.4. Seizure Analysis

In this study, only GTCS were quantified. GTCS were characterized as bilateral tonic-clonic seizures, and all were identified by visual scanning of video data by a board-certified epileptologist (CAS). Seizure duration and postictal recovery (time to resume recumbency) were compared separately for each animal, latency between lights being turned on a GTCS in B1, as well as latency between ketamine administration and GTCS in B2, were all

quantified and statistically compared with two-tailed Student t-tests ($\alpha < 0.05$). Seizure frequencies and characteristics were compared mainly between baseline and full-time therapy (2.5 mA and 24 hour stimulation).

2.5. Scalp EEG

The methods for the scalp EEG studies (Nihon-Kohden, Japan) were modified from our previous studies (Szabó et al., 2005; Szabó et al., 2013). The surface electrodes were placed according to the standard international 10–20 electrode placement system at FP1, FP2, T8, C4, Cz, C3, T7, O1, O2, A1 and A2 positions. Electrodes were also placed bilaterally beside the eyes to monitor lateral eye movements (EOG1, EOG2), over the deltoids and/or paraspinal muscles to monitor skeletal muscle activity and heart rhythm (ECG). Intramuscular ketamine (5–6 mg/kg) was used to sedate animals in order to transfer them to a primate chair for electrode placement. At these doses, ketamine can activate interictal and ictal epileptic discharges, with minimal effect on photosensitivity. Interictal epileptic discharges (IEDs) were classified as previously indicated, consisting of 4–6 Hz generalized spike-and-wave discharges (Szabó et al., 2005; Szabó et al., 2013). Seizures were classified as generalized myoclonic or tonic or bilateral tonic-clonic (Berg et al., 2010). IED and seizure rates were quantified for the 120-second sample before ILS was started. ILS was performed at least on one occasion, consisting of an initial one-minute train of 25 Hz (baboons are most sensitive to 20–25 Hz ILS frequencies), followed by five 10-second trains of 25 Hz, followed by stimulus frequency increases by 3 Hz increments between 3–30 Hz, each sustained for 10 seconds with a 5–10 second interval. Photoparoxysmal responses (PPRs) were characterized by a time-locked ictal or interictal discharge (usually within 2–3 seconds of stimulus onset) or at stimulus offset. In the first and fourth EEG studies in B1, and studies 4 and 5 in B2 (Table 2), ILS was repeated at stepwise increasing frequencies for 120–180 seconds during which the baboons were exposed to continuous VNS Therapy to evaluate its direct effect on PPRs. VNS was programmed and settings were confirmed prior to the end of the study. The electrodes were then removed and the baboons were returned to their cages. The mean duration of the scalp EEG studies was 32 (range 23 to 51) minutes.

2.6. Behavioral Testing

Both animals were formally assessed four times, once at baseline, then one month apart with the Novel Object or “Temperament Test” (Table 3). These were performed in the morning (Coleman et al., 2005), and involved the sequential presentation of two novel objects, in this case, inanimate plastic toys attached for 2 minutes to each baboon’s cage. The baboon was observed via video. Latency to touch the novel object was recorded and categorized as <10 seconds, 10–120 seconds, and >120 seconds (i.e. did not touch the object during the two minutes). Delayed engagement or lacking interaction indicate reduced attention and interest, reflecting either an impaired cognitive state or depressed affect, and which were both observed in epileptic baboons.

3. RESULTS

3.1. Seizure Outcome

Overall 60 GTCS were detected, 21 in B1 and 39 in B2 (Table 1). All of the GTCS in B1 occurred upon awakening (mean duration 50 ± 11 seconds), within five minutes from the lights being turned on. The latency between lights being turned on and seizure onset in B1 was 139 ± 84 seconds, but marginally shorter with full-time high-frequency burst VNS Therapy (105 ± 66 seconds) compared to baseline (196 ± 73 seconds) (two-tailed Student t-test, $p=0.06$). B1's GTCS evolved more often with head version or focal clonic activity lateralized more often to the right ($N=11$) than the left ($N=8$) side or without lateralization ($N=2$). Time to recumbency was 2 ± 1 minutes, but mostly associated with repetitive generalized myoclonus beginning with one minute of the end of the GTCS. B2 had 32 spontaneous GTCS (mean duration 47 ± 4 seconds), all occurring from sleep (even three seizures occurring when the lights were on). B2 rarely demonstrated any focality at GTCS onset and the time to recumbency was 4 ± 1 minutes. She continued to experience ketamine-induced GTCS (mean duration 31 ± 3 seconds) even with full-dose high-frequency VNS Therapy. The latency between ketamine administration and seizure onset in B2 was 4 ± 2 minutes, and seemed to increase from shy of 3 minutes to over 5 minutes with stimulation, though lacking statistical significance. There were no significant differences in seizure duration, and time to recumbency between VNS treatment and no-treatment periods in each baboon. While spontaneous seizure durations were similar between both baboons, time to recumbency was significantly longer in B2 than B1 (two-tailed Student t-test, $p<0.001$).

During the baseline sample, B1 experienced one GTCS per week, always within 5 minutes of the lights being turned on in the morning. Her seizure frequency was not altered for the first nine weeks of treatment, although when was treated only 6–7 am, the timing of her GTCS shifted from 7 to after midnight, related to lights being turned on by the night shift. She became seizure-free for two weeks (2.5 mA, 300 Hz signal frequency, 7 pulses, 0.5 sec IBI, 30 sec on, 1.8 minutes off) before the VNS was turned off. During the four-week pause or “wash-out” period, seizures only recurred after 14 days, with four GTCS occurring in the subsequent two weeks. High-frequency burst VNS Therapy was restarted again (2.5 mA, 4 pulses, 0.5 sec IBI, 30 sec on, 1.8 minutes off) for a two-hour window (6–8 am), and while seizures were reduced, one of her seizures again recurred when lights were turned on after midnight. For the last two weeks, once therapy was restored continuously, she became seizure-free again.

B2 had three spontaneous GTCS/week over a four-week baseline period. Once therapy was initiated, her seizure frequency decreased to one GTCS/week. In the seventh week, her seizure frequency increased unexpectedly, but it turned out that there was conflict situation with B1 at that time, in which she lost an index finger. During the six-week “wash-out” period, she started to have seizures within one week, returning to her baseline frequency by the end of this period. When VNS Therapy was restarted she had 75% reduction over a four-week period (2.5 mA, 300 Hz signal frequency, 4 pulses, 1.5 sec IBI, 30 sec on, 1.8 minutes off). This dropped to two GTCS/weeks in the final two weeks, when the current output was

decreased from 2.5 to 2 mA, in order to eliminate muscle contractions associated with the stimulus (see Adverse Effects).

3.2. Scalp EEG Outcome

At baseline, both baboons demonstrated 4–6 Hz interictal generalized spike-and wave discharges, as well as eyelid myoclonus (B1 also had generalized myoclonic seizures) on scalp EEG. Ictal or interictal epileptic discharge frequencies prior to photic stimulation were 6/min and 7/min for B1, respectively, and IED rate was 8/min for B2. Photoparoxysmal responses were noted for B1 at ILS frequencies of 3, 6, 12, 18, 25 and 27 Hz, and at 3, 6, 12, 27, and 30 Hz in B2.

Ictal and interictal discharge rates, and PPRs were not altered by chronic VNS, although there was evidence of reduced seizure severity in B1 (resolution of generalized myoclonic seizures). Brief, ketamine-related tonic seizures (10–15 seconds) were noted in studies 1–5 in B2, but not in the baseline study (this may have been a ketamine dose-dependent effect, as the baseline dose was 20% higher than in the subsequent studies). While continuous VNS stimulation for 120–150 sec did not appear to affect PPRs, stimulation for 180 sec during the fourth scalp EEG in B2 (after the wash-out period) did demonstrate a robust response across all frequencies but 21 Hz (Figure 2), as well as in the fifth study, where the number of PPRs at 25 Hz decreased from 4 to 1 of 5 trials, along with suppression of the PPR at 3 Hz. As both baboons experienced seizure reductions, high-frequency burst VNS Therapy was continued after the study period.

3.3. Behavioral Effects

Adverse effects, such as altered behavior (change in anxiety levels), appetite or sleep, were not noted, nor did the baboons exhibit any irritation (scratching or picking) in the cervical region. There were no obvious changes in vocalizations, although these were not quantifiable by video recordings. The loss of the finger in B2 occurred in the setting of a skirmish between baboons visually separated, but in adjacent cages, was not related to VNS Therapy. The Novel Object Testing revealed overall increased interest of the baboons for the objects during VNS Therapy compared to baseline or suboptimal treatment periods (Block 2 for B1, see Table 3).

3.4. Adverse Effects

There was no evidence of choking, drooling, altered respiration or appetite related to the VNS. The only side effect of high-frequency burst VNS therapy was a clinically subtle, intermittent muscle twitching affecting the neck and shoulder girdle muscles, but not the facial or back muscles, and which did not appear to bother the baboons. This artifact was not consistent, decreasing in severity with subsequent cycles of stimulation. It was noted mainly when the baboon was sedated, and not at all in wakefulness. There was subtle evidence of the twitching in sleep, but this did not lead to arousals. The twitching occurred at the same rate as the stimulation and was most visible at the 0.5 sec interburst intervals. During one study in B2, VNS stimulation parameters were altered, including current output, number of pulses, interburst interval and stimulus duration. The artifact was reduced by reduction of current output to 2 mA or stimulation duration to 21 seconds, and by increase in the

interburst interval to 1.5 sec or greater. Other beneficial physiological effects included a return of normal menstrual cycles during VNS Therapy in B2, while menstrual cycles were already regular at baseline in B1.

4. DISCUSSION

High-frequency burst VNS Therapy at 300 Hz demonstrated efficacy and tolerability in this natural nonhuman primate model of GGE. One surprising finding at baseline was that both baboons, experienced frequent weekly seizures, despite reports of only rare seizures in the past. In these two cases the seizures occurred mainly in sleep or upon awakening and may have not been observed by veterinary staff, who are mainly present during the daytime. Once high-frequency burst VNS Therapy was initiated, GTCS frequency was reduced over all VNS stimulation parameters at 2.5 mA output current. On the other hand, neither seizure duration nor postictal recovery was shortened, and the therapeutic effect wore off after 2 weeks of withholding therapy. As seizure frequency increased at lower output currents, such as 0.25 mA in B1 and 2 mA in B2, efficacy appears to depend upon current output. Shortening the treatment window in B1 to 1–2 hours around awakening, only shifted the seizure to nighttime when lights were turned on. Chronic high-frequency burst VNS Therapy did not reduce interictal or ictal discharge rates as well as photosensitivity on scalp EEG, but photoparoxysmal responses (PPRs) appeared to be suppressed by concurrent stimulation. Overall, based upon observations and behavioral testing, high-frequency burst VNS Therapy appeared to be well tolerated, with no adverse effect on temperament.

The only adverse effect was a myogenic artifact synchronized with the VNS stimuli, affecting the neck muscle and shoulder girdle. The twitches did not seem to elicit any immediate reaction in the baboons, and were not noted in wakefulness. Based upon the lack of cerebral discharges, the localized activation of muscles surrounding the vagal nerve, and the delayed activation with respect to stimulus onset (20 seconds later), it is likely that this artifact is related to the current spread along branches of the vagal nerve or the ansa cervicalis, both of which are more highly branched in the baboon, in part due to the presence of the air sacs. Nevertheless, adjustments to the current output of stimulus to 2 mA and the stimulus duration to 21 seconds eliminated the artifact acutely. Furthermore, there was evidence of habituation and resolution of the artifact even within a few cycles after programming the device. Regardless, if this stimulus artifact were to occur in humans, it may not be well tolerated. Finally, based on this study alone, we cannot extrapolate with regards to objective findings of dysphonia or hoarseness, or to subjective complaints, such as pain, muscle contractions or headaches, that have been ascribed to VNS Therapy (Ben-Menachem, 2001; Morris and Mueller, 1999).

While the exact mechanisms underlying the efficacy of VNS Therapy in the treatment of epilepsy are unknown, high-frequency burst stimulation at 100–350 Hz is thought have a more direct effect on the thalamus and cortical potentials than low frequency VNS at 20–30 Hz, while both affect excitability via brainstem structures, such as the locus coeruleus or the dorsal raphe (Alexander and McNamara, 2012; Jimenez-Rivera et al., 1987; Krahel et al., 1998). While we did not demonstrate evidence of reduction of ictal and interictal epileptic discharges in this study, the demonstration of an immediate treatment effect would have

required coordination of stimulation simultaneously with IED quantification (Kuba et al., 2002). On the other hand, continuous high-frequency burst VNS Therapy over 180 seconds appeared to reduce photoparoxysmal responses in B2 over a range of frequencies, reflecting an alteration of cortico-cortical connectivity or propagation underlying photosensitivity (Naquet and Meldrum, 1972; Mukundan et al., 2015). Lastly, while the latency of GTCS was increased by high-frequency burst VNS Therapy after ketamine administration in B2, there was an unexpected shortening of the GTCS latency from the time the lights were turned on in B1. It is possible that the shortened latency is due to an interaction between VNS Therapy and the activation of the reticular activating system, which certainly needs to be confirmed in people with juvenile myoclonic epilepsy with myoclonic seizures or GTCS upon awakening.

There were several limitations to this study, the most important one being the small number of probands and short time of testing each VNS parameter. Nevertheless, the response to initiation or withdrawal of treatment, occurred within weeks. This may either reflect a more immediate therapeutic effect than is commonly encountered with standard, low-frequency VNS Therapy, or more evident in the absence of antiepileptic medications. Other limitations may be the relatively rudimentary behavioral testing, and the discrepancy of clinical effect and EEG results, which in part, may have been mitigated by ketamine used for light sedation to apply the scalp electrodes.

5. CONCLUSIONS

In summary, high-frequency burst VNS Therapy appears to be well-tolerated and effective therapy for epilepsy. Since the epileptic baboons had not been treated with antiepileptic medications, the efficacy of this treatment cannot be extrapolated to humans with medically refractory focal and generalized epilepsies. Nonetheless, high-frequency burst VNS Therapy produces reliable effects related to the different treatment paradigms (particularly related to dose and total daily stimulation) as well as withdrawal of treatment. High-frequency burst VNS Therapy provides promising adjunctive treatment not only for medically refractory focal epilepsies, but, based on this non-human primate epilepsy model in the baboon, also for idiopathic (genetic) generalized epilepsies in humans.

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Key Points

1. High-frequency burst VNS Therapy reduced seizure frequency in two treatment-naïve epileptic baboons.
2. High-frequency burst VNS Therapy had immediate effects on photoparoxysmal responses.
3. High-frequency burst VNS Therapy was well tolerated by both baboons.
4. Cervical muscle contractions were intermittent in sedation or sleep, but controlled by reduced stimulus current or duration.

High-frequency Microburst Stimulation

For $IBI=0$, $N=1 \rightarrow$ Conventional "Tonic" VNS

For $IBI>0$, $N>1 \rightarrow$ Burst stimulation

F defines high/low frequency

Figure 1.
High-Frequency Burst Stimulation Paradigm

Figure 2.

Suppression of Photoepileptic Responses with Continuous VNS in B2. Generalized photoconvulsive responses (PCR, eyelid myoclonia) are marked by arrows in the top panels at (A1) 15 Hz and (B1) 27 Hz ILS. These PCRs are attenuated with continuous high-frequency burst VNS on the right-sided panels at (A2) 15 Hz and (B2) 27 Hz ILS. ILS onset is marked at the bottom of the page.

Table 1

VNS Therapy Settings and their Effects on **Spontaneous GTCS** Frequency

Legend: B1 & B2 denote the two baboons, sec (seconds), IBI interburst interval, wk week, GTCS generalized tonic-clonic seizure, NC no change, NA not available.

VNS Settings	Duration		Seizure Frequency	
	B1	B2	B1	B2
Pretreatment baseline	–5-0 wks	–4-0 wk	1.25 GTCS/wk	3 GTCS/wk
2.5 mA, 7 pulses, 2.5 sec IBI, 30 sec on, 5 min off	1 st wk 1 wk	1 st wk 1 wk	No GTCS	No GTCS
2.5 mA, 7 pulses, 2.5 sec IBI, 30 sec on, 1.8 min off	2 nd -3 rd wk 2 wks	2 nd -3 rd wk 2 wks	1 GTCS/wk	1 GTCS/wk
2.5 mA, 4 pulses, 1.5 sec IBI, 30 sec on, 1.8 min off	4 th wk 2 wks	4 th -5 th wk 2 wks	1 GTCS/wk	No GTCS
2.5 mA, 4 pulses, 1.5 sec IBI, 30 sec on, 1.8 min off, 6–7 am	5 th wk 2 wks	NA	1 GTCS/wk	NA
0.25 mA, 7 pulses, 0.5 sec IBI, 30 sec on, 1.8 min off	6 th -7 th wk 2 wks	NA	1.5 GTCS/wk	NA
2.5 mA, 7 pulses, 0.5 sec IBI, 30 sec on 1.8 min off	8 th -9 th wk 2 wks	6 th -7 th wk 2 wks	No GTCS	1.5 GTCS/wk
Washout period (VNS off)	10 th -13 th wk 4 wks	8–13 th wk 6 wks	1 GTCS/wk	2 GTCS/wk
2.5 mA, 4 pulses, 1.5 sec IBI, 30 sec on, 1.8 min off, 6–8 am	14 th -17 th wk 4 wks	NA	0.5 GTCS/wk	NA
2.5 mA, 4 pulses, 1.5 sec IBI, 30 sec on, 1.8 min off	18 th -19 th wk 2 wks	14 th -17 th wk 4 wks	No GTCS	1 GTCS/week
2.0 mA, 4 pulses, 1.5 sec IBI, 30 sec on, 1.8 min off	NA	18 th -19 th wk 2 wks	NA	2 GTCS/wk

Table 2

Scalp EEG Findings

Legend: IEDs interictal epileptic discharges, TST total study time, sec(onds), min(utes), IBI interburst intervals, BL baseline, mA milliamperes, EM eyelid myoclonus, GM generalized myoclonus, Y(es), N(o), NA not available or classifiable.

Study #	VNS Settings	IEDs	Seizures	ILS #	ILS Frequencies (Hz)										TST	
					25	3	6	9	12	15	18	21	24	27	30	
B1																
BL	NA	7/min	EM 6/min	1	Y	Y	Y	N	Y	N	Y	N	Y	N	30 min	
	2.5 mA, 7 pulses, 2.5 sec IBI, 30 sec, 120 sec			2	NA	N	Y	Y	Y	N	Y	Y	Y			
1	2.5 mA, 7 pulses, 2.5 sec IBI, 30 sec, 5 min	2/min	EM 6/min GM 4/min	1	Y	N	N	N	N	Y	Y	N	Y	N	26 min	
2	0.25 mA, 7 pulses, 0.5 sec IBI, 30 sec, 1.8 min	None	EM 2/min GM 1/min	1	Y	Y	Y	Y	Y	N	N	Y	Y	Y	35 min	
3	2.5 mA, 7 pulses, 0.5 sec IBI, 30 sec, 1.8 min	4/min	EM 4/min	1	Y	Y	Y	Y	Y	Y	N	Y	Y	N	25 min	
4	Off	5/min	EM 4/min GM 3/min	1	Y	Y	Y	Y	Y	N	N	N	Y	Y	51 min	
	2.5 mA, 4 pulses, 1.5 sec IBI, 150 sec			2	NA	Y	Y	Y	Y	Y	Y	N	Y	Y		
B2																
BL	NA	8/min	None	1	N	Y	Y	N	Y	N	N	N	Y	Y	22 min	
1	2.5 mA, 7 pulses, 2.5 sec IBI, 30 sec, 5 min	7/min	None	1	N	N	N	N	N	N	N	N	N	N	30 min	
2	2.5 mA, 7 pulses, 2.5 sec IBI, 30 sec, 1.8 min	2/min	EM 2/min GM 1/min	1	Y	N	N	Y	N	Y	N	Y	Y	Y	29 min	
3	2.5 mA, 7 pulses, 0.5 sec IBI, 30 sec, 1.8 min	5/min	EM 5/min	1	Y	Y	N	Y	N	N	N	NA	Y	Y	40 min	
4	Off	3/min	EM 6/min	1	Y	Y	Y	Y	N	Y	N	Y	Y	Y	39 min	
	2.5 mA, 7 pulses, 1.5 sec IBI, 30 sec, 180 sec			2	Y	N	N	N	N	N	N	NA	NA	NA		
5	2.5 mA, 7 pulses, 1.5 sec IBI, 30 sec, 1.8 min	4/min	EM 5/min	1	Y	Y	N	N	N	N	N	N	N	N	23 min	
	2.5 mA, 7 pulses, 1.5 sec IBI, 30 sec, 180 sec			2	Y	N	N	NA	N	NA	N	NA	NA	NA		

Table 3**Novel Object Test Results**

Legend: Responses at <10 seconds of presentation indicate curiosity/attentiveness, 10-2 minutes are equivocal, while >2 minutes may reflect lack of interest/attentiveness.

Baboon	Trials	Treatment	Object 1 Response	Object 2 Response
B1				
0 th wk	1	Off (Baseline)	10 sec – 2 min	No
10 th wk	2	2.5mA, 7 pulses, 0.5 IBI, 30 sec, 1.8 min	<10 sec	No
16 th wk	3	2.5mA, 4 pulses, 1.5 IBI, 30 sec, 1.8 min, 6–8 am	No	No
19 th wk	4	2.5mA, 4 pulses, 1.5 IBI, 30 sec, 1.8 min	10 sec – 2 min	10 sec – 2 min
B2				
-4 th wk	1	Off (Baseline)	< 10 sec	No
6 th wk	2	2.5mA, 4 pulses, 1.5 IBI, 30 sec, 1.8 min	10 sec – 2 min	No
11 th wk	3	Off (Washout Period)	No	<10 sec
16 th wk	4	2.5mA, 4 pulses, 1.5 IBI, 30 sec, 1.8 min	<10 sec	10 sec – 2 min