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Development of a Large Animal Model for Investigation of Deep Brain Stimulation for Epilepsy

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Key Words
Deep brain stimulation · Epilepsy · Hippocampus · Thalamus

Abstract
Background/Objectives: To better understand the mechanism of action of deep brain stimulation (DBS) for epilepsy and to investigate implantable device features, it is desirable to have a large animal model to evaluate clinical-grade systems. This study assessed the suitability of an ovine model of epilepsy for this purpose. ***Methods:*** Animals were anesthetized for surgery and 1.5 T MRIs collected. Unilateral anterior thalamic DBS leads, hippocampal depth electrodes and catheters were implanted using a frameless stereotactic system. Evoked responses and local field potentials were collected and stored for off-line analysis. ***Results:*** Despite limited neuroanatomic information for this species, it was possible to reliably implant leads into the target structures using MR-guided techniques. Stimulation of these regions produced robust evoked potentials within this circuit that were dependent on stimulus location and parameters. High-frequency thalamic DBS produced a clear inhibition of both spontaneous and penicillin-induced ictal activity in the hippocampus which far outlasted the duration of the stimulation. ***Conclusions:*** These preliminary results suggest that the sheep model may be useful for further investigation of DBS for epilepsy. The demonstration of marked suppression of network excitability with high-frequency stimulation supports a potential therapeutic mechanism for this DBS therapy.
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Introduction

Deep brain stimulation (DBS) has become an accepted medical therapy in the treatment of movement disorders such as Parkinson's disease, and is currently under investigation as a treatment for other disorders, including epilepsy. The application of brain stimulation to treat epilepsy has a long history, and many different stimulation targets have been evaluated over several decades of research [1–6]. Recently, DBS of the anterior nucleus (AN) of the thalamus has been investigated in several small pilot studies [7–9], and results of a large randomized controlled trial have been reported [10]. The rationale for the AN target comes from the early work of Mirski et al. [11–13] who demonstrated a reduction in drug-induced seizures in a rodent model with interruption, or high-fre-

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quency stimulation, of the Papez circuit. Subsequent preclinical studies [14–17] have confirmed that the AN is a key node in this pathway that can influence seizure initiation and propagation. Electrophysiologic mapping of this circuit has also been conducted in patients undergoing investigational AN DBS. Localized EEG activity in the temporal region [18, 19] and evoked potentials recorded with depth electrodes in the hippocampus (HC) [20, 21] in response to AN stimulation have demonstrated the strong connection between these structures in the human, and suggest that it is possible to modulate network activity with AN DBS [22].

The work reported here was initiated to develop a large animal epilepsy model that would allow further study of anterior thalamic DBS. In particular, to better understand the mechanism of action and to investigate potential next-generation implantable device features, a large animal model in which actual clinical systems can be chronically evaluated is required. This acute electrophysiology study was conducted to assess the suitability of an ovine model of epilepsy (penicillin injection into the HC) to address these needs, based upon the work reported by Opdam et al. [23]. Specifically, the current study assessed: (1) the ability to target and implant human-grade stimulation and recording leads into the anterior thalamic and hippocampal regions using MRI-guided stereotactic techniques; (2) reciprocal stimulation of and recording from these targets; (3) induction of ictal activity by penicillin injection, and monitoring of these regions, and (4) effects of anterior thalamic DBS on spontaneous and ictal HC neural activity.

Methods

This study was conducted at the Physiological Research Laboratory (Medtronic, Inc., Minneapolis, Minn., USA) under a protocol approved by the institutional Animal Care and Use Committee.

Surgery

Adult Polypay mixed breed sheep (n = 6; average weight 62 kg) were anesthetized with a standard regimen for surgery: morphine as premedication, followed by induction with propofol, intubated and maintained on >2% isoflurane, with a fentanyl/ketamine drip. Following anesthesia, 1.5 T MRIs were collected and transferred to a surgical planning station (Stealth Station Treon, Medtronic, Inc.). Trajectories for unilateral thalamic DBS leads (Model 3389, Medtronic, Inc.), HC depth electrodes (Model SD04R-SP05X-000, Ad-Tech, Inc.), and HC catheters (Intramedic PE-20 tubing) for penicillin delivery were calculated based on gross anatomic descriptions of the ovine brain [24]. No current stereotactic atlas was available for this species. Devices were implanted using a frameless stereotactic system (NexFrame, Medtronic, Inc.) following similar procedures used for human DBS surgery [25]. The thalamic DBS leads were 1.27 mm in diameter with four 1.5-mm-long electrode contacts spaced 0.5 mm apart. Typical impedance for adjacent bipolar electrode pairs was approximately 1,000 Ω. The HC leads were 1.1 mm in diameter with four 2.41-mm-long electrode contacts, separated by 5 mm (typical impedance 500 Ω). Contacts were denoted as numbers 0 (deepest) to 3 (most superficial) on both leads and each contact could be independently programmed for stimulation or recording. Hippocampal catheters with an inner diameter of 0.38 mm were implanted 2.0 mm adjacent to the HC lead on a parallel trajectory, with the tip approximately adjacent to contact 1. Following the surgical procedure, anesthesia was modified according to the protocol reported by Opdam et al. [23] (high-dose morphine and acepromazine) in order to minimize suppression of cortical neural activity.

Stimulation and Recording

Electrical stimulation was delivered to the implanted leads via a custom-designed system based upon the electronics of an implantable DBS pulse generator (Activa system, Medronic, Inc.). Local field potential (LFP) and evoked potential (EP) recordings from both leads were acquired using Grass P511 amplifiers, digitized with the BioPac MP150 system (BioPacSystems Inc., Goleta, Calif., USA), and stored for off-line analysis. For EP measurements, trains of stimuli at 5 Hz were presented for 30 s. Each adjacent pair of contacts on the thalamic lead was tested to determine the bipolar combination that produced the largest hippocampal EPs. This 'best' bipolar anode/cathode configuration was then used for subsequent testing of stimulation effects on LFPs and ictal activity with stimulus bursts from 5 to 160 Hz presented for periods ranging from 1 to 40 s. Pulse width was fixed at 120 μs.

Penicillin (8,000 U/0.1 ml) was slowly delivered via the hippocampal catheters using a Hamilton syringe or syringe pump. Multiple doses were delivered in some animals in order to assess the level and type of ictal activity produced.

Data Analyses

Following the procedure, animals were euthanized and postmortem MRIs collected to verify lead position. In two early animals, gross pathology was conducted to assess lead placement and confirm agreement with MRI localization; no histology was conducted. Data were analyzed using Acqknowledge 4.0 software (BioPac Systems). EPs were averaged off-line by selecting approximately a 10-second period of maximal, stable response, and using the stimulus artifact as the trigger. Spontaneous activity levels in LFPs were quantified by measuring the root mean square amplitude and/or calculating power spectral density over a specified time window.

Results

Surgical Targeting

Despite limited neuroanatomical atlas information for this species, it was possible to reliably target and implant electrodes into the anterior thalamic and hippocampal

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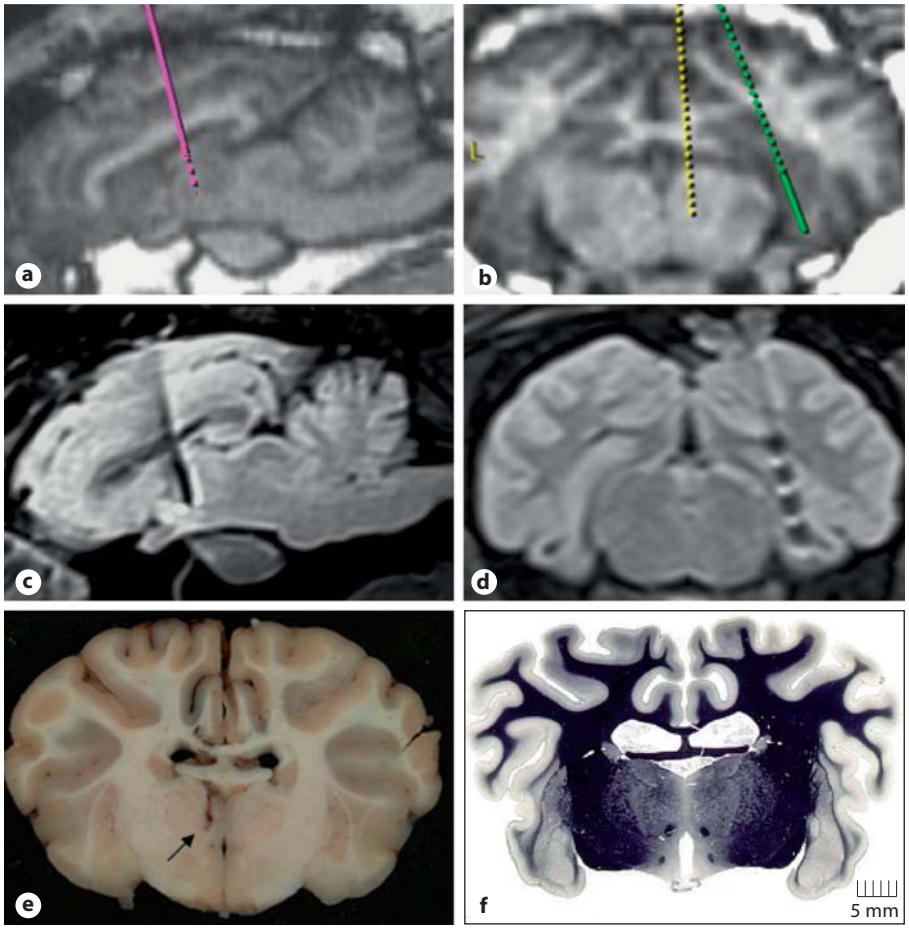

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Fig. 1. Surgical targeting: preoperative surgical plans (**a**, **b**) and postoperative MR images (**c**, **d**) showing a DBS lead placed in the anterior thalamic region (**c**) and a depth electrode placed in the HC (**d**). Gross pathology slide (**e**) showing a DBS lead track (arrow) and the corresponding atlas slice (**f**) for reference. The tail of the caudate can be seen at the lateral edge of the ventricles immediately adjacent to the fornix; the anterior nuclei lie at the dorsal surface of the thalamus.

regions using MRI-based stereotactic techniques. Figure 1 shows representative preoperative surgical planning and postoperative lead localization images for the thalamic and hippocampal leads. The bottom panels also show a gross pathology image from the same animal illustrating the location of the thalamic lead track and a corresponding atlas image for comparison.

Postoperative MRIs were analyzed for all animals to assess lead positions. Placement into the anterior thalamic region was confirmed in 5 of 6 animals, with one trajectory too posterior, behind the mid-commissural point. In each animal, the thalamic electrode contact (cathode) that generated the largest hippocampal evoked response was identified. For deeper lead placements (as in fig. 1), the more proximal contacts were found to generate the largest HC EPs, while for more shallow placements, the 0 and 1 contacts were typically selected. The average (±SD) position for the center of these contacts (n = 5) was: X = 3.2 (±1.3) mm; Y = 2.6 (±1.6) mm; Z = 4.0 (±0.9) mm (relative to the mid-commissural point). The average (±SD) anterior commissure to posterior commissure (AC-PC) length in this group (n = 6) was 14.3 (±0.3) mm. Figure 2 shows this stereotactic point illustrated on typical preoperative planning images, indicating a position consistent with a location in the anterior thalamus. In the sheep, the anterior nuclei lie at the rostral and dorsal pole of the thalamus, spanning a volume of approximately 3–4 mm laterally, 3–4 mm in the anterior-posterior plane, and 2–3 mm from the surface of the thalamus [26]. This region is located directly below a large fornix (relative to human anatomy), separated by 1 mm or less of lateral ventricle. The close proximity, and large size of the fornix in this species, which contains direct afferent and efferent hippocampal fibers, complicated the interpretation of the neural recordings as described below. Placement of recording leads into the HC, a large, easily visualized cortical region, was highly repeatable with the methods employed.

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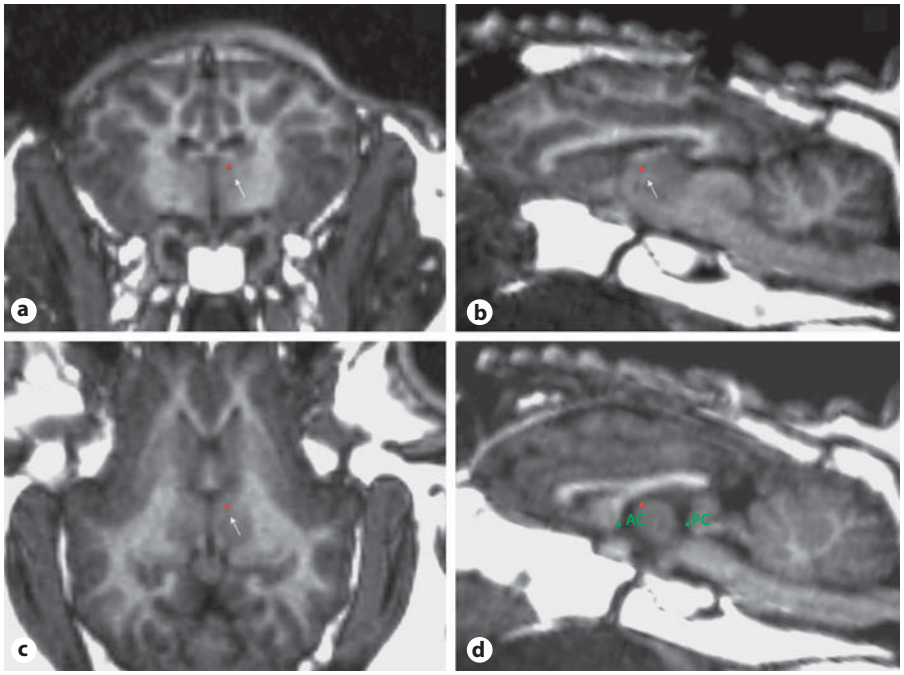

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Fig. 2. Surgical targeting: location of average stereotactic coordinates (dot) in the coronal (**a**), sagittal (**b**), and axial (**c**) planes, corresponding to the average position of the DBS cathodal contact that generated the largest hippocampal EPs. **d** Sagittal section at midline showing AC-PC and corresponding midline position of this point.

Hippocampal-Evoked Responses

Stimulation of the anterior thalamic region produced EPs in the HC that varied depending upon the electrode contacts selected and the stimulation parameters. The evoked responses were sometimes visible in the raw LFP signal and exhibited a temporal 'waxing and waning' over the 30-second stimulus burst (fig. 3a). Responses were generally largest on the deepest HC contacts (maximum amplitudes were approximately 1.5 mV), and exhibited polarity reversal between different bipolar recording channels, demonstrating the specific, local origin of the dipole source (fig. 3b). The latency for this evoked response in the HC ranged between approximately 30 and 50 ms. Figure 3c shows the graded nature of the HC EPs generated by increasing the stimulus intensity on a fixed electrode pair. These EPs were also sensitive to the stimulation frequency. Figure 3d shows representative EPs to three different stimulation frequencies (5, 10, 20 Hz). As the frequency was increased, the amplitude of the HC EPs decreased considerably. At higher frequencies, there was virtually no EP present; however, these responses were difficult to assess as the interstimulus interval (and stimulus artifact) approximated the response latency.

The latency and morphology of the HC-evoked response differed depending upon the electrode contacts stimulated. To investigate possible sources of this variability, a mapping sequence was conducted in one animal by advancing the DBS electrode in 1-mm increments and recording the HC EPs generated by a fixed stimulus on a single contact pair. These results are shown in figure 4 with insets of an atlas slice and the postoperative MRI showing the final lead position for reference. As the electrode was advanced toward the target, the first EP recorded in the HC had a latency of approximately 25 ms. With further advancement, the morphology of the EP shifted to a dual-peaked response with the appearance of a longer-latency (50 ms) component. With stimulation at deeper locations, the shorter-latency response became less prominent and then disappeared. At a depth of approximately 5–6 mm below the site of initial EP generation, no HC EPs were elicited.

Anterior Thalamic Region-Evoked Responses

For HC stimulation, evoked responses recorded by thalamic electrodes showed a complex multiphasic morphology, with a longer-latency (40–50 ms) component and multiple synchronous short-latency (10–20 ms) peaks. Figure 5 shows a series of graded responses generated by bipolar HC stimulation at increasing intensities. The largest responses recorded by the thalamic lead were typically generated by stimulation of the deepest HC electrode contacts and were on the order of 100–200 μV. The general morphology of these responses was consistent across the different recording channels and between animals.

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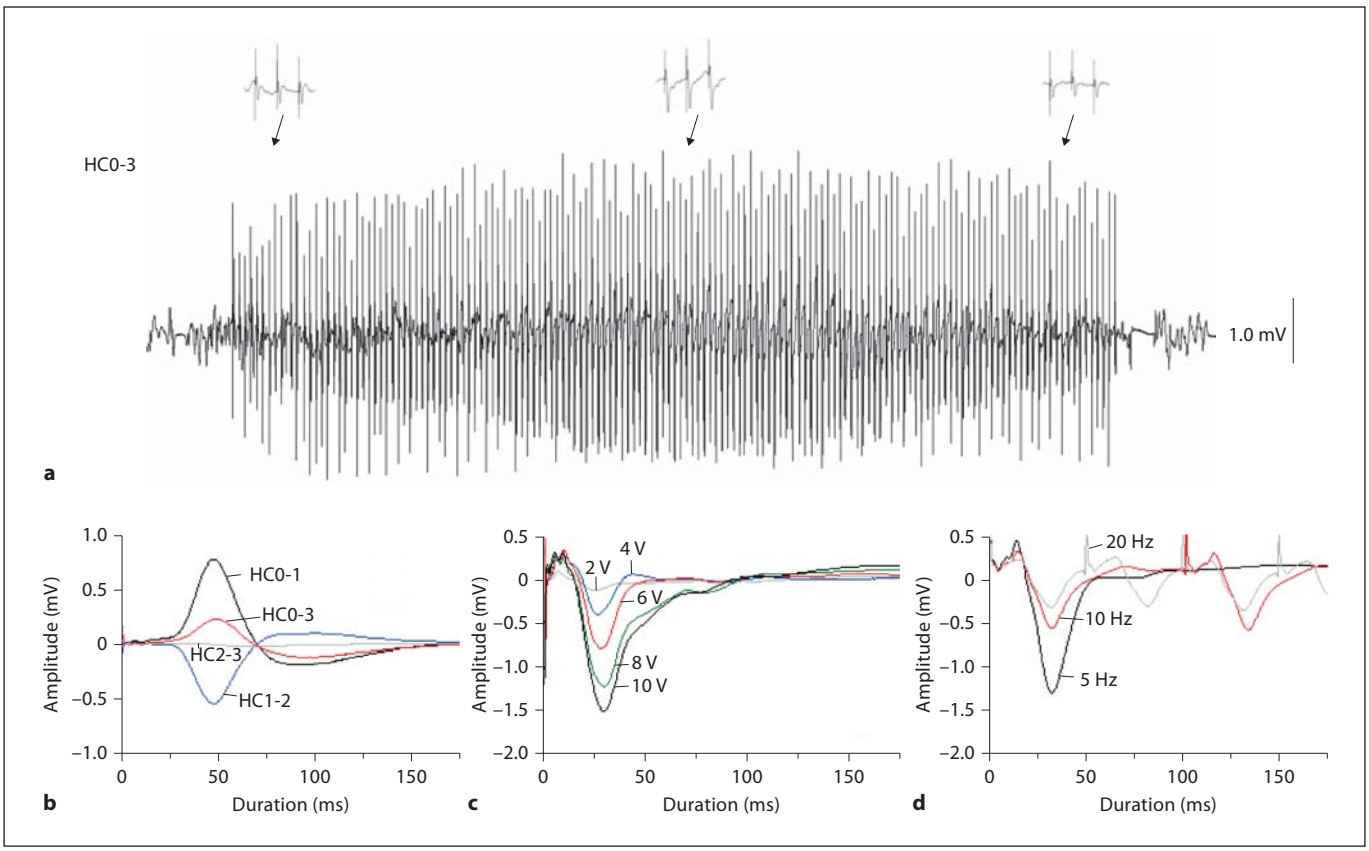

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Fig. 3. Hippocampal EPs. **a** Raw LFP response to 30-second anterior thalamic stimulation (6 V) burst illustrating change in individual EP amplitudes during the burst (variation in stimulus artifact amplitude is due to sampling rate resolution as shown in the insets). **b** Averaged EPs recorded from different HC electrode pairs illustrating polarity reversal. **c** Averaged EPs elicited by different stimulation amplitudes showing graded input-output response. **d** Averaged EPs elicited by different stimulation burst frequencies.

Anterior Thalamic Stimulation – Effects on Spontaneous Activity

The effects of different frequencies of anterior thalamic stimulation on hippocampal spontaneous activity (LFPs) were also examined. Figure 6a shows an example of the changes in HC spontaneous activity in response to 10-second bursts of stimulation across a range of frequencies. Low-frequency stimulus bursts had little effect on the level of spontaneous activity (top trace). As the burst frequency was increased above 40–80 Hz, there was a clear reduction in the level of spontaneous activity, which lasted well beyond the stimulus burst. The second trace shows the same paradigm, with the order of the burst frequency reversed, from high (160 Hz) to low (5 Hz), again demonstrating the suppression of HC spontaneous activity by high-frequency, but not low-frequency DBS. These results are quantified in figure 6b, which shows the change from baseline in spontaneous LFPs measured in both the HC and the thalamus for these two examples. The changes in the level of spontaneous activity measured in the thalamus paralleled those observed in the HC suggesting a widespread suppression reflected across the network. In both examples, the level of spontaneous activity recovered from its minimum, induced by the high-frequency bursts, over a period of several minutes. Figure 6c shows the power density spectra of HC LFPs measured in 4 animals at two time points in the test paradigm illustrated in figure 6a: prior to stimulation and following the final 160-Hz burst. In all animals, there was a marked reduction in the amplitude of the HC LFP power spectrum after the delivery of the high-frequency DBS bursts.

To further investigate the time course of the recovery phenomenon, a second test paradigm was employed. In

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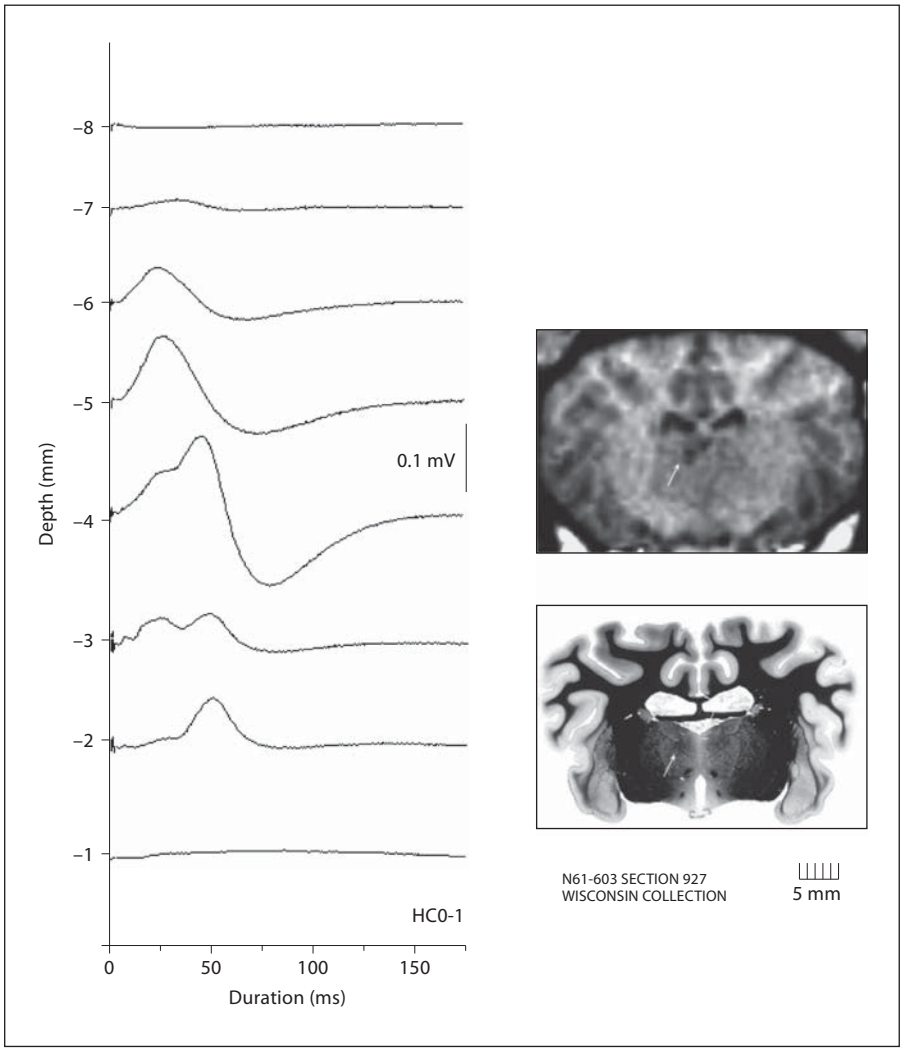

Fig. 4. Hippocampal EPs to AN region stimulation at different locations. Averaged EPs to stimulation (6 V, contacts 0–, 1+) at different depths along the planned DBS lead trajectory are shown. The postoperative MRI illustrating the final position of the DBS lead tip, and a representative atlas slice are shown for reference (arrow indicates approximate lead tip position).

this case, high-frequency (160 Hz) bursts of different durations were delivered, and the level of HC spontaneous activity assessed for return to baseline. As illustrated in figure 7, short burst durations (1 and 2 s) produced only a small reduction in spontaneous activity, which recovered quickly. A 5-second burst produced a maximal level of suppression (approx. 45% of baseline), which recovered over a 3- to 4-min period. Longer burst durations did not result in a greater level of suppression, but did extend the recovery period to approximately 6–7 min.

Administration of Penicillin

Injection of penicillin into the HC resulted in the production of characteristic spike and wave activity (fig. 8a) as previously described in several species [27]. Spiking was first observed on the electrode contact closest to the catheter tip (in this example HC2) and then on other HC channels, often with reversed polarity, indicating the local generation of the source activity. The amplitude and frequency of the spiking typically increased over the first minutes following administration and then stabilized into a low-frequency (<1 Hz) regular pattern. Different doses of penicillin produced very similar effects, the main difference being the frequency of the induced spiking. In no case did this spiking progress to a higher-frequency bursting pattern, or self-terminate over a period of hours.

This spiking activity could also be observed, following some variable delay, on some recording channels of the thalamic lead, as illustrated in the top traces of figure 8a. There was considerable variability in the reflection of the HC spiking in the thalamic recordings that appeared to be related to the level of ongoing spontaneous activity.

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Spiking in the thalamus was most clearly observed in situations where the level of asynchronous background activity was low relative to the amplitude of the spikes. This was observed both across animals and within the same animal as the spontaneous activity level varied over time during the ictal period.

The effects of thalamic stimulation on penicillin-induced ictal activity were also assessed. Figure 8b illustrates these effects on hippocampal spiking following the test paradigm shown earlier. During and after each 10-second burst of stimulation, the largest amplitude spiking was suppressed. The spiking then resumed following a variable delay, beginning with smaller-amplitude spikes with a different morphology, and progressing to the amplitude and shape of the original spikes. In this example, the suppression of ictal activity did not show the clear frequency dependence observed for the spontaneous activity, with all burst frequencies producing a similar reduction in spiking.

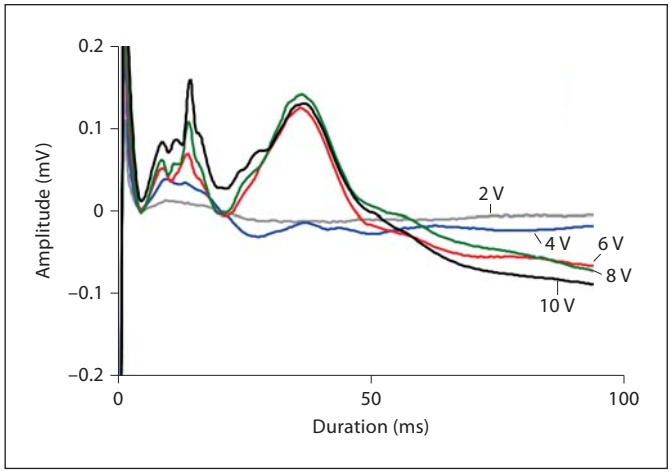

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Fig. 5. Evoked responses recorded on the thalamic DBS lead to stimulation in the HC at different amplitudes (HC contacts 0–, 1+).

Discussion

Stereotactic surgery in this species was greatly facilitated by the use of MR-based surgical planning tools, and a frameless delivery system. The available neuroanatomic information for the sheep is fairly limited [24, 26] and does not provide AC-PC-based measurements for stereotaxy. It was therefore necessary to develop standard, easily visualized landmarks that could serve as reference points relative to the targeted structures. In the axial plane, the midline junction of the pineal body and superior colliculi at the level of the third ventricle provided a reliable anatomic landmark across animals. The dentate gyrus of the HC lies immediately lateral to this point and is readily visible in an MR image. At this axial level, the anterior nuclear region is also visible bilaterally, as the most anterior extent of the thalamic mass. In the sheep, the anterior thalamus is bounded rostrally and superiorly by a very large fornix which lies on the dorsal surface of the thalamus. This large white matter structure extends posteriorly and transitions directly into the dorsal gyrus of the HC.

Although the sheep represents a large animal model, the brain size is still small compared to the human (approx. 10% by weight) and the electrodes used were therefore relatively large for the targeted neural structures. This was more of an issue for the anterior thalamus, where the DBS lead was quite large compared to the neural target. Analysis of lead contact locations and relationship to HC-evoked responses did, however, indicate an average active contact position within the anterior thalamic region. Based on postoperative imaging, gross pathology, and atlas measurements, it is likely that only 1 or 2 contacts of the DBS electrode were within the AN region with a well-targeted placement, with the more dorsal contacts located at the level of the fornix.

The results of stimulation in this region were consistent with this anatomic structure. During the mapping procedure, the first HC responses observed had a short latency, and appear to result from activation of the direct afferent pathway through the fornix. As the DBS electrode was advanced, the resultant EP exhibited a dual-peaked morphology, with the appearance of a second, longer-latency peak. Finally, deeper placement of the stimulating electrode generated only the longer-latency potential, with the early peak absent. This later response peak appears to reflect activation of the anterior thalamic target. Its longer latency is consistent with the Papez circuit pathway, which courses through the cingulate gyrus before terminating in the HC.

The HC evoked responses to low-frequency stimulation may represent the local correlate of the recruiting rhythm recorded on scalp EEG with thalamic stimulation in humans [8, 18, 28]. The buildup of these EPs with repetitive stimulation and little or no response to the initial stimulus are similar to that classically described as key features of the recruiting response.

Evoked responses recorded in the AN region to HC stimulation were also consistent with the anatomic struc-

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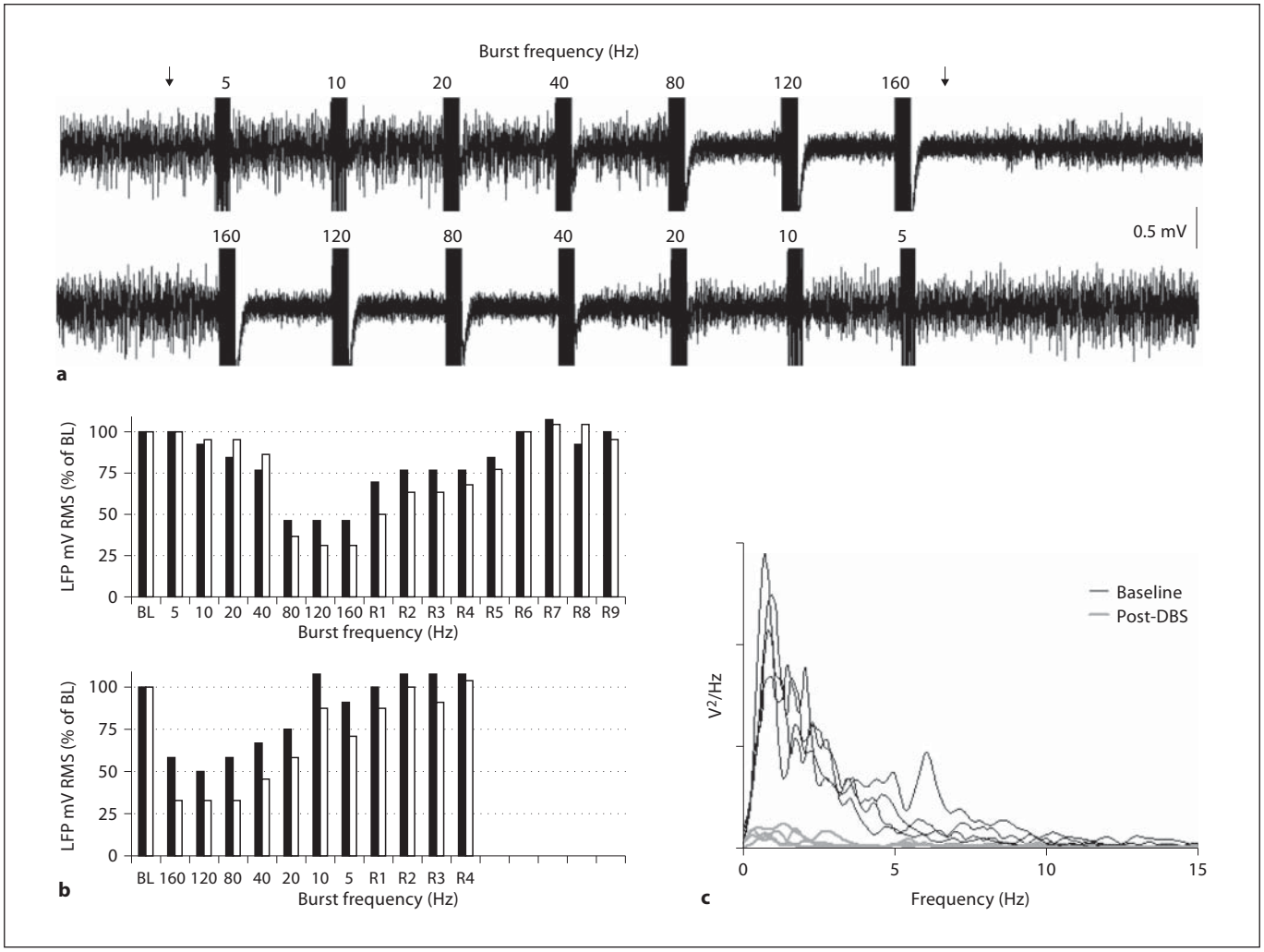

Fig. 6. Effects of different DBS stimulation burst frequencies on LFPs recorded in the HC. **a** Response to low-/high-frequency (top) and high-/low-frequency (bottom) burst patterns (10-second burst, 1 per minute). **b** Level of spontaneous activity measured in the HC (solid) and AN (open) for the two examples shown in **a** (recovery period R1, R2, etc. represent 1-min intervals following final stimulation burst). **c** Power spectral density of LFPs (n = 4, first subject not tested; one excluded due to lead placement) at two time points (arrows) of the paradigm shown in the top trace of **a**. Absolute amplitudes across animals were normalized to comparable baseline levels for display. BL = Baseline; RMS = root mean square.

ture and known circuit pathways. These EPs exhibited a multi-peaked shorter-latency (10–20 ms) component which appeared highly synchronous, suggesting propagated activity from HC efferents, and possibly antidromically activated afferents, through the white matter of the fornix. This early response was followed by a longer latency (approx. 40 ms), more diffuse potential, which could reflect subsequent activation of the thalamus.

In the one animal with the posterior lead placement, the HC response to stimulation differed notably in one respect. In this case, stimulation did produce short-latency (20–30 ms) responses in the HC; however, higher-frequency stimulation repeatedly triggered afterdischarges, even at very low stimulation amplitudes. This was the only animal in which afterdischarges were observed. Based upon postoperative imaging and atlas estimates, active contact location in this animal was at the posterior portion of the fornix as it transitioned into the dorsal HC.

Penicillin injection into the HC resulted in a slow progression of ictal activity, dominated by high-amplitude,

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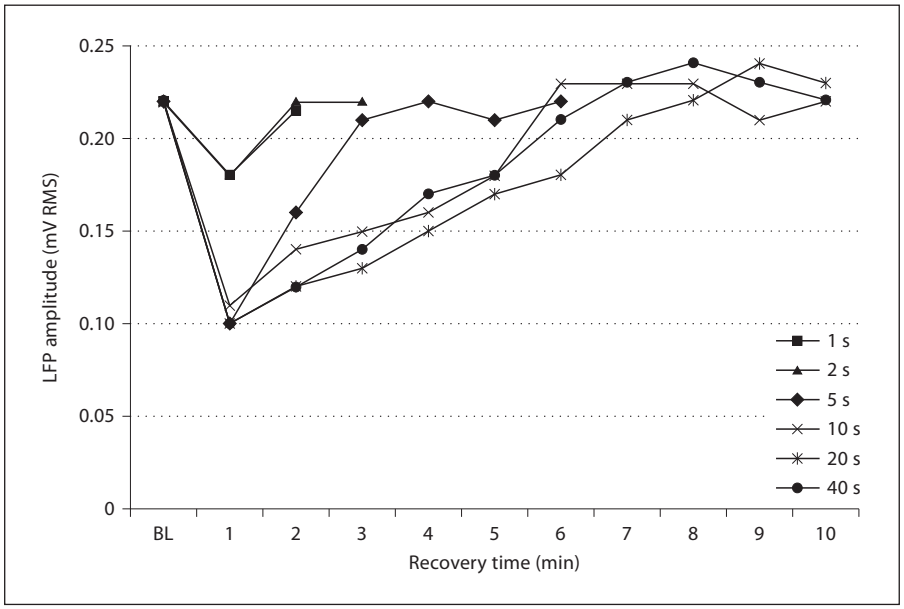

Fig. 7. Recovery of spontaneous activity levels in the HC following different burst durations of high-frequency (160 Hz) thalamic stimulation. BL = Baseline; RMS = root mean square.

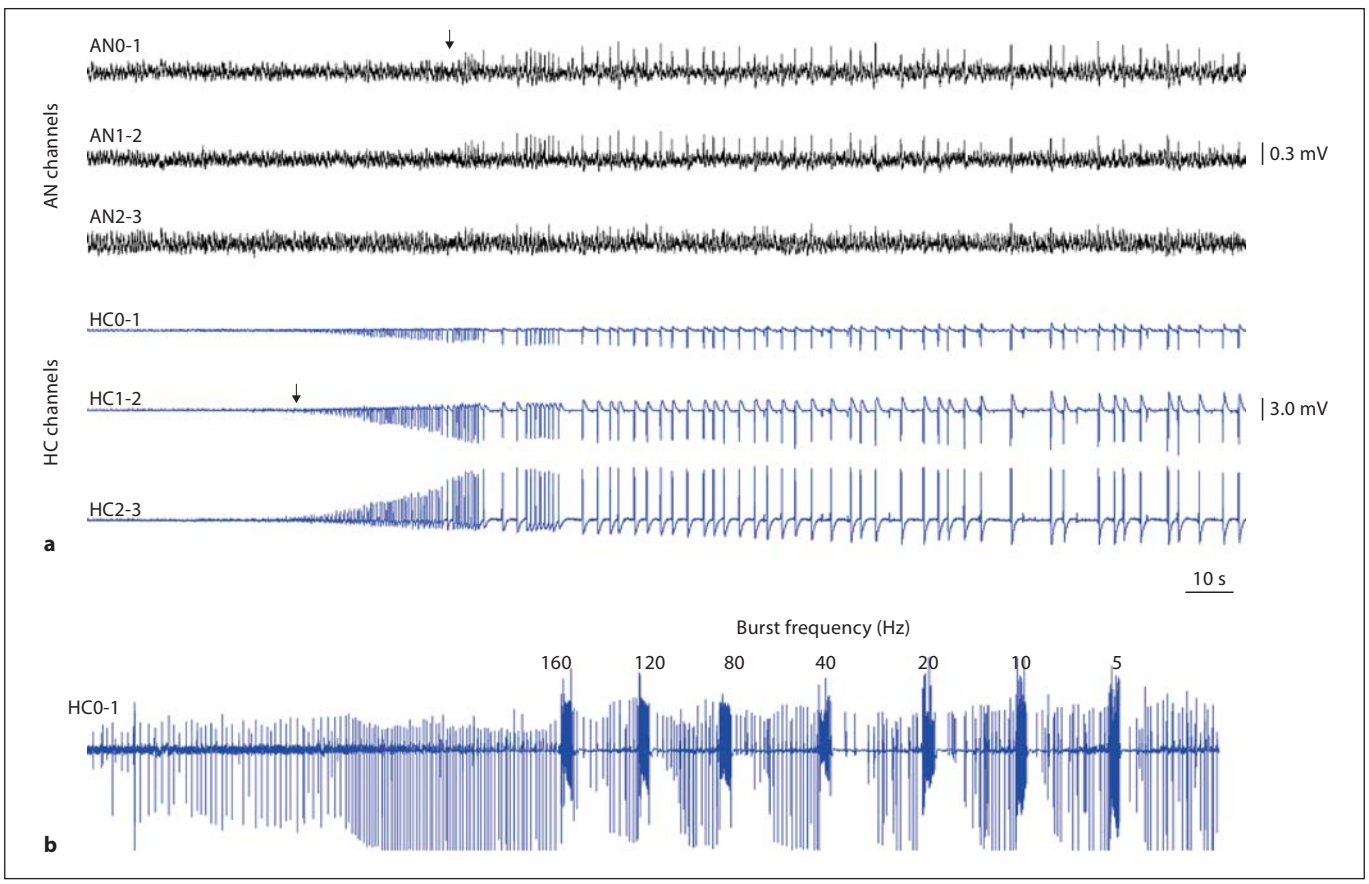

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Fig. 8. Administration of penicillin. **a** Epileptiform activity recorded in the HC (bottom three traces) and AN region (top three traces) following injection of penicillin. Spiking was first recorded in the HC near the injection site (bottom arrow) and soon thereafter reflected in some channels of the AN recordings (top arrow). **b** Effects of different burst frequencies of thalamic stimulation (10-second burst) on penicillin-induced spiking recorded in the HC illustrating suppression of HC spiking.

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low-frequency spike and wave generation, that remained relatively constant, and did not evolve to higher-frequency activity, even with high doses. In all animals, ictal activity originating in the HC was also reflected in the thalamic recordings. This activity was recorded differentially with closely spaced electrodes, appeared on only some channels within the AN region, and showed a latency difference between the HC and AN spikes that was consistent with the evoked response measurements. Based upon these considerations, we conclude that this activity was of local origin and not far-field reflections of HC spiking. These results suggest that recordings from DBS electrodes in the anterior thalamus may afford the opportunity to monitor seizure activity originating in remote brain regions.

A key finding of this study was the effect of high-frequency thalamic DBS on the spontaneous neural activity within the HC. The background level of this neural 'noise' can be considered as a measure of the overall state of cortical and network excitability. Thalamic region stimulation produced a clear, repeatable, and frequency-dependent suppression of HC excitability, which lasted for several minutes following cessation of stimulation. Low-frequency (5 Hz) stimulation appeared to result in a facilitation, evidenced as an increase in individual EP amplitudes from the onset of the stimulation burst, and had no apparent effect on the level of spontaneous activity. Higher stimulation rates resulted in a reduction in the amplitude of the EPs, as well as a suppression of spontaneous activity with frequencies above 40 Hz. Stimulus rates similar to those used for DBS therapies resulted in a marked and prolonged suppression of cortical activity, suggesting that high-frequency afferent stimulation acts to increase hippocampal inhibitory tone.

Thalamic region DBS also produced a reduction in penicillin-induced ictal activity in the HC, although the effect was less pronounced, and showed less frequency dependence, than the effect on spontaneous activity. The proconvulsant effect of penicillin is known to be mediated via inactivation of $GABA_A$ cortical interneurons, resulting in glutamate-driven synchronous firing of pyramidal neurons [29]. The fact that stimulation still produced a suppressive effect in the presence of high doses of penicillin suggests that this GABA network could still be activated by DBS and/or that these effects are mediated via other pathways.

From the perspective of DBS therapy for epilepsy, these finding are very intriguing. First, the clear demonstration of suppression of hippocampal excitability by high-frequency DBS provides a very plausible mechanism of action for the therapy, and supports the clinical findings that have been reported [10]. Modulation of cortical excitability is the goal of numerous pharmacotherapy approaches to treat epilepsy, and studies have suggested that levels of cortical excitability are directly correlated with the occurrence of seizures [30, 31]. Molnar et al. [32] demonstrated changes in motor cortex excitability in patients with AN DBS using a paired-pulse trans-cranial magnetic stimulation paradigm. Frequency-dependent mesial temporal inhibition was also demonstrated in 1 patient with AN DBS [22], although the time course differed from that reported here. This mechanism of action is also consistent with the results reported by McCracken and Grace [33, 34] who described inhibition of orbital-frontal cortical neurons by high-frequency DBS in a different thalamocortical pathway. These authors also noted a widespread, synchronized inhibition measured across cortical and subcortical networks, as demonstrated here by the parallel changes observed in thalamic and HC spontaneous activity following stimulation. On the other hand, the DBS-induced suppression of hippocampal activity also raises questions regarding effects on learning and memory. Clinical evaluations have not documented neurocognitive deficits in patients treated with AN DBS [7–10]; however, self-reported memory impairment was noted to be higher in the active versus control group in the recent study of Fisher et al. [10].

Second, the duration of cortical suppression, which far outlasted the period of stimulation, provides a rationale for use of a cycled pattern of therapy (e.g. 1 min on, 5 min off) as used clinically. The underlying mechanism of this prolonged depression of neural activity is unclear. The time course is inconsistent with a purely GABA-mediated phenomenon which would not be expected to persist for minutes following a short stimulus burst. The reduction of penicillin-induced ictal activity also argues against a predominantly GABAergic mechanism. These results are also not congruent with long-term synaptic depression, which is most commonly associated with sustained low-frequency (e.g. 1 Hz) stimulation [35], as opposed to brief, high-frequency bursts which generated the most pronounced effects observed here. The long time course and frequency dependence appear to be more consistent with a neuromodulatory mechanism, possibly acting via the adenosine pathway. Adenosine is a potent modulator within the central nervous system, including the HC, and has been shown to inhibit glutamate transmission via a presynaptic mechanism [36]. Its role in producing inhibitory tone in several brain circuits is well documented [37]

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and levels of extracellular adenosine are directly related to neuronal metabolic activity [38].

Cunha et al. [39] reported that levels of adenosine in hippocampal slices were increased by high-frequency, but not low-frequency stimulation, and recently, the involvement of adenosine in the therapeutic mechanism of thalamic DBS has been suggested [40, 41]. The marked suppression and slow recovery of both spontaneous and ictal activity following high-frequency DBS bursts are consistent with this type of metabotropic regulatory mechanism; however, direct evidence is not provided by our results. Additional studies to examine the neurochemical changes that parallel these electrophysiologic observations could help to clarify the fundamental basis of these prolonged effects induced by DBS.

This study had several limitations that should be noted. Although the number of animals is small, the sample size is consistent with large animal investigations and early model development. Not unexpectedly, placement of leads into the anterior thalamic region varied as experience was gained over the course of the study, with the greatest variability occurring in the AP dimension. Also, due to the size of the DBS electrode relative to the sheep thalamus, and the close approximation of the fornix in this species, it is possible that even with a well-placed lead, stimulation may have activated both afferent pathways into the HC depending upon the stimulation parameters and contacts selected. The contact pairs used to assess the effects on spontaneous LFPs were those that generated the largest EPs in the HC, which could have involved contributions from both pathways. Even though the narrow (0.5 mm) spacing between adjacent contacts on the DBS lead results in a very restricted stimulation field, as shown in the mapping experiment, it is not possible to solely attribute the results to AN stimulation. However, the frequency-dependent effects on hippocampal LFPs were highly repeatable and robust within and across animals, and suggest that high-frequency DBS of these pathways into the HC produces similar effects.

Conclusions

In summary, these preliminary results support the use of this sheep model for further investigation of implantable devices for the treatment of epilepsy. The demonstration of marked suppression of hippocampal activity with high-frequency stimulation suggests a potential therapeutic mechanism of DBS for epilepsy that is consistent with current theories of DBS network effects. This model affords the opportunity for future study of DBS mechanisms in this thalamocortical network, and the potential to explore clinically relevant questions such as the contribution of microlesion effects, and the influence of stimulation parameters on normal and ictal activity in both acute and chronic recordings.

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Disclosure Statement

All authors are employees of Medtronic, Inc.

References

1 Cooper IS: Effect of chronic stimulation of anterior cerebellum on neurological disease. Lancet 1973;7796:7206.

2 Velasco F, Velasco M, Ogarrio C, Fanghanel G: Electrical stimulation of the centromedian thalamic nucleus in the treatment of convulsive seizures: a preliminary report. Epilepsia 1987;28:421–430.

3 Benabid AL, Minotti L, Koudsie A, de Saint Martin A, Hirsch E: Antiepileptic effect of high-frequency stimulation of the subthalamic nucleus (corpus luysi) in a case of medically intractable epilepsy caused by focal dysplasia: a 30-month follow-up: technical case report. Neurosurgery 2002;50:1385–1392.

4 Chkhenkeli SA, Chkhenkeli IS: Effects of therapeutic stimulation of nucleus caudatus on epileptic electrical activity of brain in patients with intractable epilepsy. Stereotact Funct Neurosurg 1997;69:221–224.

5 Velasco M, Velasco F, Velasco AL: Centromedian-thalamic and hippocampal electrical stimulation for the control of intractable epileptic seizures. J Clin Neurophysiol 2001; 18:495–513.

6 Upton AR, Cooper IS, Springman M, Amin I: Suppression of seizures and psychosis of limbic system origin by chronic stimulation of anterior nucleus of the thalamus. Int J Neurol 1985;19–20:223–230.

7 Hoadie M, Wennberg RA, Dostrovsky JO, Lozano AM: Chronic anterior thalamus stimulation for intractable epilepsy. Epilepsia 2002;43:603–608.

8 Kerrigan JF, Litt B, Fisher RS, Cranstoun S, French JA, Blum DE, Dichter M, Shetter A, Baltuch G, Jaggi J, Krone S, Brodie M, Rise M, Graves N: Electrical stimulation of the anterior nucleus of the thalamus for the treatment of intractable epilepsy. Epilepsia 2004;45:346–354.

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9 Andrade DM, Zumsteg D, Hamani C, Hodaie M, Sarkissian S, Lozano AM, Wennberg RA: Long-term follow-up of patients with thalamic deep brain stimulation for epilepsy. Neurology 2006;66:1571–1573.

10 Fisher R, Salanova V, Witt T, Worth R, Henry T, Gross R, Oommen K, Osorio I, Nazzaro J, Labar D, Kaplitt M, Sperling M, Sandok E, Neal J, Handforth A, Stern J, DeSalles A, Chung S, Shetter A, Bergen D, Bakay R, Henderson J, French J, Baltuch G, Rosenfeld W, Youkilis A, Marks W, Garcia P, Barbaro N, Fountain N, Bazil C, Goodman R, McKhann G, Babu Krishnamurthy K, Papavassiliou S, Epstein C, Pollard J, Tonder L, Grebin J, Coffey R, Graves N, SANTE Study Group: Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. Epilepsia 2010;51:899–908.

11 Mirski MA, Ferrendelli JA: Interruption of the mammillothalamic tract prevents seizures in guinea pigs. Science 1984;226:72–74.

12 Mirski MA, Fisher RA: Electrical stimulation of the mammillary nuclei raises seizure threshold to pentylenetetrazol in rats. Epilepsia 1994;35:1309–1316.

13 Mirski MA, Rossell LA, Fisher RA: Anticonvulsant effect of anterior thalamic high frequency electrical stimulation in the rat. Epilepsy Res 1997;28:89–100.

14 Hamani C, Ewerton FI, Bonilha SM, Ballester G, Mello LE, Lozano AM: Bilateral anterior thalamic nucleus lesions and high-frequency stimulation are protective against pilocarpine-induced seizures and status epilepticus. Neurosurgery 2004;54:191–195.

15 Hamani C, Hodaie M, Chiang J, del Campo M, Andrade DM, Sherman D, Mirski M, Mello LE, Lozano AM: Deep brain stimulation of the anterior nucleus of the thalamus: effects of electrical stimulation on pilocarpine-induced seizures and status epilepticus. Epilepsy Res 2008;78:117–123.

16 Takebayashi S, Hashizume K, Tanaka T, Hodozuka A: Anti-convulsant effect of electrical stimulation and lesioning of the anterior thalamic nucleus on kainic acid-induced focal limbic seizure in rats. Epilepsy Res 2007;74:163–170.

17 Takebayashi S, Hashizume K, Tanaka T, Hodozuka A: The effect of electrical stimulation and lesioning of the anterior thalamic nucleus on kainic acid-induced focal cortical seizure status in rats. Epilepsia 2007;48:348–358.

18 Zumsteg D, Lozano AM, Wennberg RA: Rhythmic cortical EEG synchronization with low frequency stimulation of the anterior and medial thalamus for epilepsy. Clin Neurophysiol 2006;117:2272–2278.

19 Zumsteg D, Lozano AM, Wieser GW, Wennberg RA: Cortical activation with deep brain stimulation of the anterior thalamus for epilepsy. Clin Neurophysiol 2006;117:192–207.

20 Zumsteg D, Lozano AM, Wennberg RA: Depth electrode recorded cerebral responses with deep brain stimulation of the anterior thalamus for epilepsy. Clin Neurophysiol 2006;117:1602–1609.

21 Osorio I, Overman J, Giftakis J, Wilkinson SB: High frequency thalamic stimulation for inoperable mesial temporal epilepsy. Epilepsia 2007;48:1561–1571.

22 Zumsteg D, Lozano AM, Wennberg RA: Mesial temporal inhibition in a patient with deep brain stimulation of the anterior thalamus for epilepsy. Epilepsia 2006;47:1958–1962.

23 Opdam HI, Federico P, Jackson GD, Buchanan J, Abbott DF, Fabinyi GC, Syngeniotis A, Vosmansky M, Archer JS, Wellard RM, Bellomo R: A sheep model for the study of focal epilepsy with concurrent intracranial EEG and functional MRI. Epilepsia 2002;43:779–787.

24 Vanderwolf, CH, Cooley RK: The Sheep Brain: A Photographic Series, ed 2. London, Kirby Co, 2002.

25 Holloway KL, Gaede SE, Starr PA, Rosenow JM, Ramakrishnan V, Henderson JM: Frameless stereotaxy using bone fiducial markers for deep brain stimulation. Neurosurgery 2005;103:404–413.

26 Johnson JI, Sudheimer KD, Davis KK, Kerndt GM, Winn BM: The Sheep Brain Atlas (online). East Lansing, Michigan State University, 2009.

27 De Deyn PP, D'Hooge R, Marescau B, Pei YQ: Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsants. Epilepsy Res 1992;12:87–110.

28 Dempsey EW, Morison RS: The production of rhythmically recurrent cortical potentials after localized thalamic stimulation. Am J Phys 1942;135:293–300.

29 Anderson MI, O'Mara SM: Analysis of recordings of single-unit firing and population activity in the dorsal subiculum of unrestrained, freely moving rats. J Neurophysiol 2003;90:655–665.

30 Badawy R, Macdonell R, Jackson G, Berkovic S: The peri-ictal state: cortical excitability changes within 24 h of a seizure. Brain 2009;132:1013–1021.

31 Wright MA, Orth M, Patsalos PN, Smith SJ, Richardson MP: Cortical excitability predicts seizures in acutely drug-reduced temporal lobe epilepsy patients. Neurology 2006;67:1646–1651.

32 Molnar GF, Sailer A, Gunraj CA, Cunic DI, Wennberg RA, Lozano AM, Chen R: Changes in motor cortex excitability with stimulation of anterior thalamus in epilepsy. Neurology 2006;66:566–571.

33 McCracken CB, Grace AA: High-frequency deep brain stimulation of the nucleus accumbens region suppresses neuronal activity and selectively modulates afferent drive in rat orbitofrontal cortex in vivo. J Neurosci 2007;27:12601–12610.

34 McCracken CB, Grace AA: Nucleus accumbens deep brain stimulation produces region-specific alterations in local field potential oscillations and evoked responses in vivo. J Neurosci 2009;29:5354–5363.

35 Massey PV, Bashir ZI: Long-term depression: multiple forms and implications for brain function. Trends Neurosci 2007;30:176–184.

36 Wu C: Possible role of glutamatergic neurotransmission in regulating ethanol-evoked brain ascorbate release. Neurosci Lett 1994;171:105–108.

37 Dunwiddie TV: The physiological role of adenosine in the central nervous system. Int Rev Neurobiol 1985;27:63–139.

38 Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW: Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. Science 1997;276:1265–1268.

39 Cunha RA, Vizi ES, Ribeiro JA, Sebastião AM: Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. J Neurochem 1996;67:2180–2187.

40 Bekar L, Libionka W, Tian GF, Xu Q, Torres A, Wang X, Lovatt D, Williams E, Takano T, Schnermann J, Bakos R, Nedergaard M: Adenosine is crucial for deep brain stimulation-mediated attenuation of tremor. Nat Med 2008;14:75–80.

41 Tawfik VL, Chang SY, Hitti FL, Roberts DW, Leiter JC, Jovanovic S, Lee KH: Deep brain stimulation results in local glutamate and adenosine release: investigation into the role of astrocytes. Neurosurgery 2010;67:367–375.

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