Reviewer Comments:

Reviewer #2 (Comments to the Authors):

This is a very thorough and novel proof-of-concept investigation in epilepsy research. The authors have modeled neuronal spiking data recorded from 24 neurons from the hippocampus of an epilepsy patient to reconstruct a neuronal network (RNN) that can faithfully reproduce them. Spontaneous seizure activity was then initiated in the RNN by modifications in two of its parameters (B and sigma). Thereafter, this simulation model was used as an in-silico testbed for designing and testing efficient neurostimulation patterns for seizure abatement.

The significance of having such a testbed for different stimulation schemes for seizure abatement is very important. Furthermore, this study's conjectures are critically important and could explain the underperformance of the currently available neuromodulation schemes in seizure abatement. The study addresses important unanswered questions in neurostimulation and, together with the authors' cautious remarks about its shortcomings, deserves to be published so that the respective scientific community takes notice.

We strongly thank the reviewer for his support of this work and his recommendation for its publication

What is missing in this investigation to become more clinically translational in the future is the development of a similar methodology through appropriate optimal spatial neurostimulation at preictal points so that seizures are not even initiated. The difficulty in such an endeavor would be the detection of the preictal state. The authors acknowledge the research by a few groups in this direction. Important contributions in the literature should be added. My more detailed observations and suggestions to the authors follow:

1.   Line 49: Add the following reference to synchronicity:
•   L. Good, S. Sabesan, S. Marsh, K. Tsakalis, D. Treiman & L.D. Iasemidis, "Control of synchronization of brain dynamics leads to control of epileptic seizures in rodents", Int. J. Neural Systems, vol. 19, No. 3, 173-196, 2009.

RESPONSE: This paper is highly relevant to our work & the citation has been added

2.   Line 52: Designing optimal neurostimulation patterns: Reference to closed-loop adaptive neurostimulation to be added here for completion reasons:
•   N. Chakravarthy, K. Tsakalis, S. Sabesan & L.D. Iasemidis, "Homeostasis of brain dynamics in epilepsy: a feedback control systems perspective of seizures", Annals of Biomedical Engineering, vol. 37, pp. 565-585, 2009.
•   N. Chakravarthy, S. Sabesan, L.D. Iasemidis & K. Tsakalis, "Controlling epileptic seizures in a neural mass model", J. Combinatorial Optimization, vol. 17, pp. 98-116, 2009.
•   N. Chakravarthy, S. Sabesan, L.D. Iasemidis & K. Tsakalis, "Controlling synchronization in a neuron-level population model", Int. J. Neural Systems, vol. 17, pp. 123-138, 2007.

RESPONSE: These citations have been added and a sentence has been added referencing closed-loop neurostimulation in the introduction. Furthermore, these citations have been added to the section dealing with responsive neurostimulation.

3.   Line 118: "...thereby allowing the (filters) interactions between two pyramidal cells to be inhibitory..."

changed

4.   Line 157: "...seizure and control states..." Better replace "control states" with "non-seizure states" or "nonictal states" throughout the manuscript to avoid confusion with your control scheme.

This is a great point. We have adopted the terminology of 'seizure & non-seizure states'

5.   Line 160: "2 different modes of stimulation..."

changed

6.   Line 179: "...control FMR": same problem as in "control states". Better say "reference FMR"

great point. The terminology of 'reference MFR' has been applied

7.   Line 201: "than"◊ "then"

changed

8.   Line 212: "18/24 neuronal models' ◊ "18 out of 24 neurons"

changed

9. Figure 1 caption:
a) Correct as: "...effectively disconnected from the population...".

changed

b) "Notice that as 1/λ is increased (and thus regularization is weakened)" But regularization strength is ~ 1/λ? Correct.

RESPONSE: Regularization is actually proportional to lambda. Thus as lambda→inf, all coefficients approach 0 (see equations A6 and A7 and surrounding text).

10. Lines 244-246: "It was found that raising the baseline by B = 30% relative to the threshold and lowering σ to .725 was sufficient to generate spontaneously emerging realistic seizures lasting anywhere between a few seconds to over a minute": Why and how were the "seizures" terminated spontaneously in the model? (In their statement here, the authors give the impression that they could control the duration of seizures without a stimulation input.)

RESPONSE: Indeed, by changing the aforementioned parameters, the network developed two stable states (i.e. bistability). The noise injected into the system caused the network to spontaneously transition between the 2 stable states. This is akin to a 2-state markov chain. Only after these parameters were fixed, did we attempt to 'customize' the system input to abate seizures. To clarify this, the following sentence has been added:

> "We emphasize that the network would transition between the seizure and non-seizure states randomly and without any manual intervention, akin to a two-state Markov chain. "

See also point below about future work.

11. **Lines 291-293**: "Interestingly, 2 of the selected electrodes (22 and 24) stimulated the epileptic subnetwork (Fig. 3e), while the other two stimulated outside the subnetwork, suggesting that direct stimulation of the seizure focus itself may not be the most effective route for seizure abatement". First, how consistent was this result across seizures? Second, it would be worthy to report how those electrodes behave (differently than the others?) at the initiation of seizures in the model.

RESPONSE: Given the seizure initiating parameters used in the paper (see previous point), the seizures were highly stereotyped: all seizures involved the same set of active and quiet cells. Furthermore, although the paper does not delve into the temporal specifics of how seizures are initiated (e.g. which cells fire first), by examining multiple randomly initiated seizures, we have found that their initiation is also largely stereotyped (e.g. the same

cells tend to start firing first). In future work, we plan on exploring this point in much more detail in both patient-based and synthetic neuronal networks.

12. Lines 402-403: "However, in PD"◊ "The advantage in controlling PD is that ...."

Changed to: "However, when dealing with PD neurostimulation, one has …"

13. Line 405: "physician must oftentimes wait several months before they can access the..." -> "physicians must oftentimes wait several months before they can assess the..."

changed

14. Line 410: "prevent the seizure from ever occurring". For historical reasons, before Neuropace and other groups that work to prevent seizures from ever occurring, a research group had introduced the idea of responsive stimulation. They called this control scheme feedback control stimulation. The references are:
•   Iasemidis & Tsakalis, "Pacemaker for treating physiological system dysfunction", US Patent 2009-0264952-A1, 10/22/09.
•   K. Tsakalis, N. Chakravarthy, S. Sabesan, L.D. Iasemidis & P.M. Pardalos, "A feedback control systems view of epileptic seizures", Cybernetics Systems Analysis, vol. 42, pp. 483 -495, 2006.
•   K. Tsakalis & L.D. Iasemidis, "Control aspects of a theoretical model for epileptic seizures", Int. Journal of Bifurcations and Chaos, vol. 16, pp. 2013-2027, 2006.

RESPONSE: these are very relevant citations. They have been added to the section of the discussion dealing with more sophisticated epilepsy control strategies (section 4.2)

15. Line 434: "Furthermore, it was observed that the optimal stimulation" -> "Furthermore, it was observed that our proposed optimal stimulation"

changed

Reviewer #3 (Comments to the Authors):

I must say that I started reading this paper and anticipated that I would be extraordinarily skeptical by the end. ¬On the contrary, I must applaud the authors, in that they have dealt with what I think are almost all of the likely criticisms of this work, and present a powerful and compelling exposition.

In brief, they record from 24 neurons from the CA3 region of a human epileptic hippocampus, use machine learning to reconstruct a network from these data, and then after pruning to create a sparse network, develop a set of optimized stimulation parameters that could potentially be incorporated into a responsive stimulator such as the Neuropace device.

From a machine learning perspective, this is a true tour-de-force. I find it extremely annoying that one might be able to one day dispense with learning much neuroscience if dumb black box machine models might just supplant supposed human expertise. But as with the best of machine learning applications in biology, there is deep neuroscience knowledge that lies behind this particular black box approach. I am impressed that the authors are offering their code in the manuscript. This is one study where you could never replicate the science in finite time without the code, and I would strongly recommend that a condition of the publication is that there is a durable link provided by the authors to this code, and a data sample, or that the same content is archived at the journal with this paper. You could teach a full semester course on how to perform the calculations of this paper, and the prerequisites would be daunting for a neuroscientist.

It is of course ridiculous that one might use depth recorded single cell data in this fashion. It presupposes that prior to implantation you already know that your electrodes have circumscribed enough of the relevant neurons

within or without the focus so that they will be effective in stimulating the epilepsy into submission. And it supposes that the microwires involved would be effective many years. But the authors have dealt with some of this well in the discussion. **Could epilepsy control ever be based on single neuron stimulation? Obviously I do not know, but my impression is that the answer is no given present technology for many practical reasons. I would suggest that the authors make this more emphatic in their discussion of the issue (or disagree with me - their choice).**

RESPONSE: We largely agree with the reviewer. Stable single units are difficult to record due to cell death, neuronal drift, lead mechanical movements, etc… Furthermore, real-time spike sorting, while feasible, is quite challenging. The study was done with single units due to the historical focus of the lab on single unit modeling / system identification. Future work will aim to extend the framework to continuous signals. The section discussing this point has been expanded to take a stronger position on this issue:

> "The model presented here relied on single unit activity. In practice, however, recording and sorting stable single units is quite difficult (Gilja et al., 2012; Dhawale et al., 2017). Thus, in future work we shall apply the presented framework for identifying optimal stimulation to continuous electrophysiological signals such as ECoG. In this case, each of the specific steps would be modified, while the overall framework would remain the same. For example, in the simulated annealing algorithm the current MFR based cost function would need to be substituted for one which can be applied to continuous signals, such as one based on high frequency oscillations."

We typically place depth electrodes in hippocampi to confirm that all seizures are emanating from just one hippocampus, or if the rest of the seizure data are discordant. Typical temporal lobe epilepsy with structural changes of mesial temporal sclerosis, does not require depth electrode study prior to recommending resection for concordant patients. Placing microwire electrodes into heavily damaged sclerotic hippocampus is probably not the best option. This study suggests that one might then need to consider targeting the afferent pathways to such epileptic structures - a strategy not well articulated in the literature.

There is a tacit assumption in the paper that if you can record from a neuron at a point in the volume, that you can equivalently stimulate it from that same point. Although such reciprocity has never been proven, until it is, this is quite a leap of faith. **I would suggest adding this reciprocity issue in the discussion so that the community interested in this subject would think about this.**

RESPONSE: We fully agree with the reviewer. In fact, upon further researching this point, we found evidence against this reciprocity. Work by Histed et al., 2009 (Neuron) used calcium imaging to show that electrodes record spikes from adjacent neuronal soma, while they preferentially stimulate axons of neurons whose soma may not be anywhere near the electrode. This is another fundamental issue of using this approach with spikes rather than continuous signals (see point above). We have added the following sentence to be more transparent about this issue:

> "Additionally, there is evidence that the neurons an electrode records and those which it stimulates are not equivalent (Histed et al., 2009)."

The quality of the writing is excellent. I searched for anything I can find regarding typos, and mostly have found a few annoying suggestions for clarity such as:

• Figure Legend 4: There are more than 1 type of red line. Specify as 'dotted' the 'red lines in (D-G)'

corrected
• In the last sentence of Page 9, 'Neither PTs or RTs,...', clarify this to be sure that the sentence refers clearly to synchronized PI and RT. Otherwise it looks like the sentence refutes the results of the paper.
corrected

• In Figure legend 4A, it looks like 4 rather than 2 seizure events to me. No two epilepsy experts ever agree on how many seizures fit on a page of EEG, but at the least don't call this tracing 2.

RESPONSE: the reviewer has correctly pointed out a typo. It should indeed be 4 seizures.

• Page 13, clarify the sentence "However, neither of these stimulus styles performed as well as random unsynchronized stimulation over multiple sites", so that it is not read as a refutation of all of the results in the paper.

RESPONSE: corrected to read "However, neither of these synchronized stimulus styles performed as well as random unsynchronized stimulation…"

I find the Appendix to be superb. And it is phenomenally annoying in that there is very little biophysics required to seek the results of the paper. My opinion is that the short-term memory of the strategy employed (< 100 ms) will be a deficit that needs to be addressed in future work. This is not to say that a reconstructed network focusing on short term causality, or even short-term perturbations in an epileptic network as proposed in the paper, will not be accurate. But longer-term temporal processes and their time constants are introduced in real neuronal networks for several reasons, one of which is that ionic concentration gradients are built up over relatively long time scales, and both the buildup and dissipation of these gradients are a central facet to seizure onsets. So if you wanted to be predictive, rather than just responsive to seizure onsets, such critical longer time scale effects will need to be incorporated into the modeling.

In summary, I find this an important and potentially seminal work in this arena. I see no reason not to sign this review.

Steven Schiff

Steven, I am extremely grateful and flattered by your emphatic support for this work. It is certainly not everyday one gets such a positive review. I should also say that I am a huge fan of your pioneering work in bringing control theory to neuroscience and DBS. Would love to chat about how those ideas could be used to extend this work.

-Roman
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