Ms. No. CLINPH-D-14-7886
The Human Burst Suppression Electocenphalographram of Deep Hypothermia
Clinical Neurophysiology

Dear Editors and Reviewers,

Thank you for the thoughtful comments on our manuscript. Below please find a point-by-point summary of the revisions we have made to address these comments. We hope that you will find the revised manuscript acceptable for publication.

Best regards,

M. Brandon Westover and Patrick L. Purdon for the authors

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Editorial Office comments:

1. The separate abstract-file should contain only the Abstract; remove everything else (as this belongs to the manuscript-file).

2. Your "Summary Statement": this is not something we publish. Please remove.

We have removed the “Summary Statement”.

3. The Highlights should be attention-grabbing so that the reader feels that this paper is a "must read". Your Highlights (1, 2) are too long.

We have shortened highlights (1,2).

4. You can remove the "AUTHOR CONTRIBUTIONS".

Done.

5. You provide one legend for Figures 1-3. Please submit three separate (different) legends.

Done.

6. (See also the next item.) Please quote all figures in the correct order. You refer to figures 8, 4, 1-3, 5, 7, 8.

Done.

7. NB: Also refer to Figure 6.

Done.

8. File of Figure 2A-G: you can almost read the words "Case 1" (I guess). Please remove from this file.

Done.

Reviewer #2: *In this small prospective cohort of anesthetized patients undergoing deep hypothermia for cardiac surgery, the authors studied the effect of hypothermia on the EEG, and specifically on the pattern of suppression-burst. They report that lowering body temperature to mid and deep hypothermia is initially associated with the appearance of the suppression-burst pattern and then with a progressive decline in amplitude and duration of the bursts.*

1. The observation that deepening hypothermia is associated with progressive electrical cerebral inactivity ("suppression") is not new but the study brings interesting quantitative information that might prove useful for the development of quantitative, and more reliable, approaches for intra-operative monitoring, as aptly discussed by the authors.

Thank you.

2. From a methodological standpoint, the quantification of suppression (which seems a more appropriate term to me than depth of burst-suppression) is remarkably done, if a bit redundant. Why resort to three different metrics (BSP, burst duration and suppression duration)? Based on the data presented in Figures 4 and 5 one would expect that they would be highly correlated. Is this the case? Which one correlates best with temperature?

As shown in Figure 4, the overall relationship in the BSP vs temperature data can be captured reasonably well by fitting a straight line. However, because BSP is the probability of the EEG being in the suppressed state at any given moment, in principle an increase in BSP can occur because either bursts become briefer, suppressions

become longer, or some combination of both. This is the reason that we chose to evaluate all three quantities. As shown in Figure 5, it turns out that with increasing temperatures bursts durations tend to increase exponentially, while suppression durations exponentially decrease toward zero.

To clarify these points and the rationale for analyzing the temperature dependence of all three metrics, we have edited and added text to lines 408-412 of the revised Results section.

3. A small semantic note: I believe it is incorrect to refer to a pattern as burst-suppression if the time spent is suppression is shorter than "bursts"; these recordings are better described as containing "episodic low amplitude events" (see Brenner Dis Nerv Syst 1975 for a discussion on this subject). This should be clarified in the manuscript.

We have clarified this terminological point in the paragraph of the Methods section where we introduce the BSP, lines 302-308 of the revised manuscript.

My main concern with the manuscript relates to the quantification of the spectral content of bursts (spectral morphology is a cute term but one that is not widely used or accepted) and the interpretation that they authors chose to give to their findings. Changes in spectral content of the bursts, while minimized by the authors appear not only real but significant to me. From the graphs in Figure 8, it appears that even in the population average (Figure 8H) and despite (1) the wide variability across patients, as detailed at length by the authors, and (2) the relatively small number of recordings, there is a statistically significant difference between spectra measured in the mid and deep hypothermia conditions. From the same figures, one can observe differences in the order of at least 3dB. This indicates that there is at least a two-fold increase or decrease in power in some frequency bands (a decrease in the beta band and an increase in the delta, theta and alpha bands). The authors consider changes of this magnitude to be of little, if any, biological significance, to the point of stating in several places in the manuscript that the spectral morphology remains stable with decreasing temperature. This is surprising to me as changes of a similar magnitude (a two-fold increase or decrease) are of major functional significance in other settings. An important example is the phenomenon of event-related synchronization and desynchronization where functional activation of a cortical area, arguably not a trivial event, is accompanied by spectral changes that are typically of the same order of magnitude (two-fold decrease or increase - and this is using intracranial recordings!-; see Crone at al., Brain 1998, 121 : 2301 for instance) as the ones observed by the authors with deepening hypothermia. In addition, the fact that the scalp EEG offers only a limited

view on the spectral content of brain activity, with activity above 30Hz being difficult, if not impossible, to reliably assess, should also be acknowledged by the authors. This limitation prevents them to draw any conclusion on changes in activity in the gamma band and higher and should lead them to tone down their conclusions on the stability of the spectral content of bursts with deepening hypothermia.

We use the term ‘spectral morphology’ to refer specifically to the shape of the spectrum. We use this term here instead of 'spectral content' because our data indicate that while the total spectral power of bursts differed substantially between different temperature conditions, these differences were relatively small after normalizing for total power. ‘Spectral content’ is a more general term that refers to all spectral differences, including not only the location of any spectral peaks, but also the overall/total power.

With respect to the reviewer's main point, we agree that any discussion about possible underlying mechanisms based on our data should be regarded as tentative, and have revised the discussion section to emphasize the speculative nature of our comments. In addition, we acknowledge the limitations of scalp EEG for investigation of brain activity in the beta, gamma, and higher frequency ranges.

We have added a "limitations" subsection to the Discussoin emphasizing both the tentative nature of our comments on mechanisms and the need for further fundamental research to clarify the nature and mechanistic underpinnings of burst suppression at low temperature (lines 581-628 of the revised manuscript).

5. An additional source of concern is the use of anesthetics. While this is of course unavoidable from a clinical standpoint, the influence of anesthetics on hypothermia-induced EEG changed and vice versa should be acknowledged by the authors. Is it possible that the only effect of hypothermia is to render the brain more susceptible to the effect of isoflurane or propofol? As a side note, there are some reports of EEG changes in accidental hypothermia; none of them have reported the occurrence of a burst-suppression pattern. In particular, cases with moderate hypothermia, at which the authors observe the onset of suppression-burst, do not show suppression-burst, suggesting that this pattern is in (great) part due to anesthesia. Also, in a similar study cited by the authors (Stecker at al. 2001a), suppression-burst was reached at much lower temperature (24.4+/-4°C vs. 30.9+/-5.2°C). Why this discrepancy? Could it be due to a difference in the definition of suppression-burst (vide supra) or to a difference in the infusion rate of isoflurane (0.4% vs. 1%)? Additionally, I would like to know if anesthetic levels were measured and remained stable during the induction and the maintenance of deep hypothermia. If not stable, when did rates and levels of anesthetics change compared to changes in temperature and EEG? This is of major importance to

understand the respective contribution of temperature and anesthetics on EEG changes.

We agree that the specific temperature thresholds at which the onset of burst suppression and ECI occur, and the quantitative relationship between BSP and temperature, are undoubtedly influenced by the presence of Isoflurane (n=10) and propofol (n=1) anesthetic administration throughout hypothermia.

Isoflurane levels were measured before and after the onset of deep hypothermic circulatory arrest (DHCA), but could not be measured while patients were on bypass (n=10). Propofol levels were not measured (n=1). Nevertheless, expired isoflurane was kept constant in all cases at 1% before bypass, and both propofol and isoflurane administration rates were kept constant throughout bypass. Thus it is unlikely that the strong and systematic temperature dependence of burst suppression characteristics results from random drift in serum anesthetic levels during bypass.

We have added a paragraph to the “limitations” section of the Discussion to make these important points (lines 594-628 of the revised manuscript).

6. Finally, given the limitations discussed above and additional points presented below, I am not convinced that the presented data support in any way the theoretical model put forward by the authors. There is a wealth of information on the effect of hypothermia on neuronal activity. Hypothermia has profound depressant effects on ion transport and synaptic transmission, both involved in the genesis of the EEG signal. (See for instance Yang XF. et al J Physiol 567:215-224 or Eight and Erulkar Nature 1976.) This is in contrast with the effect of hypothermia on ATP levels themselves, which are stable, if not slightly increased, due to a reduction in both ATP consumption and synthesis (the latter slightly less than the former ; see Erecinska et al. 2003 for a review on the subject). Given the known dependence of the burst-suppression pattern on glutamate neurotransmission (see Lukatch et al. 2005) and on the activity of a class of pacemaker cortical interneurons (see Deuchars et al. 1994), it seems to me that an alternate model based on the disruption of neurotransmission and cortical networks activity is at least as likely as a model based on energy depletion. This alternate model has the advantage to account for all the observations presented in this manuscript, including the likely changes in the spectral content of bursts.

We agree that our results do not provide direct evidence for the hypothesis that suppression dynamics are mediated by effects on ATP synthesis rates as opposed to some other substrate or a combination of substrates. Liley et al also recently pointed out in reference to our earlier modeling work that while existing evidence does indeed seem

to favor the general “fast-slow” dynamical mechanism that we advocate, it is possible that more than one biophysical mechanism produces the same final dynamical behavior seen in burst suppression in different circumstances (Front Comput Neurosci. 2013 Apr 30;7:46).

We have added text and citations to highlight alternative possible biophysical mechanisms (lines 483-507 of the revised manuscript). We have also rewritten this section of the Discussion to emphasize that the essential features of our proposed extension of the “fast-slow” model for burst suppression are at the descriptive level of dynamical-systems, rather than at the level of biophysical mechanisms. It is quite possible that the dynamical model that we propose could be realized by more than one of the known biophysical processes involved in burst suppression.

7. Minor points include

- the excessive number of figures, due to the redundancy of Figure 1-3 (two of them could be presented as supplemental material) and of Figure 4-6 (same comment)

We would prefer to leave these figures in the main text if space permits. We respectfully defer to the editor for instructions on this point.

8. - the use of the term "blip", which has no accepted definition in electroencephalography (although widely used in radar detection and brodcasting, although each time with a different meaning) ; I am certain the authors will find an adequate term for simply configured bursts in Noachtar et al. 1996 or in Hirsch et al. 2013

We have removed the term “blip”. We have replaced it with the more descriptive phrase, “simple, low-amplitude slow waves” (lines 429-445 of the revised manuscript).

9. - the presence of multiple intermediate peaks in the cumulative density function of suppression duration at 31°C, 27°C. Could the authors comment on this? It seems that the effect of temperature on the degree of suppression is not monotonic. Unless there is a change in anesthetic regimen at these points?

There was no change in anesthetic regimen at these points. The multiple peaks in the upper panels of Figure 5 are probably not a "real" finding, and likely result from insufficient data to produce completely smooth CDF estimates. For this reason we chose to fit these data with the monotonic models shown in the lower panels of Figure 6. We have added a comment to the Results section commenting on this point (lines -408-418 of the revised manuscript).

Reviewer #3: *This is an interesting manuscript assessing outcome in adults with postanoxic coma, using EEG the burst suppression ratio (BSR). The authors found that BSR had higher sensitivity, specificity, positive prediction value, and negative prediction value in predicting poor outcome in adults with postanoxic coma than EEG grading and amplitude-integrated EEG patterns.*

We appreciate the reviewer's remarks.