Author’s response to reviews

Title: Long-term effects of cranial irradiation compared with intrathecal chemotherapy in treatment of childhood leukemia: A MEG study of power spectrum and correlated cognitive dysfunction

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Author’s response to reviews: see over
Dear Dr. Scott,

Thank you for the opportunity to revise our manuscript, which is hereby resubmitted. We would also like to thank Dr. Kleen and Dr. Butz for their valuable reviews. We appreciate their comments very much and believe they helped us to improve the manuscript substantially.

Below, we will reply their comments point-by-point.

**Regarding Dr. Kleen's review:**

*Major recommendations*

Ad 1) Although Daunorubicin (an anthracycline) is mostly known for its cardiotoxicity, it has been demonstrated to be neurotoxic to laboratory rats by increasing CNS levels of nitric oxide (Joshi P, Vig PJ, Veerisetty V, Cameron JA, Sekhon BS, Desaiah D: *Increase in brain nitric oxide synthase activity in daunorubicin-treated rats*. Pharmacol Toxicol 1996, Feb;78(2):99-103). But this neurotoxic mechanism does not differ from the mechanism associated with e.g. methotrexate (MTX), which also increases CNS levels of nitric oxide and is well-known for its neurotoxicity (Uzar E, Sahin O, Koyuncuoglu HR, Uz E, Bas O, Kilbas S, Yilmaz HR, Yurekli VA, Kucuker H, Songur A: *The activity of adenosine deaminase and the level of nitric oxide in spinal cord of methotrexate administered rats: protective effect of caffeic acid phenethyl ester*. Toxicology 2006, Feb;218(2-3):125-33). MTX was used in both the CT+CRT group and the CT-only group. We cannot think of any other mechanism through which daunorubicin could interact with CRT in a way that none of the other chemotherapeutic agents could.

Ad 2) Smaller head size was not measured directly, but meeting with these patients, it was visible with the naked eye. Also, the average smaller head size was implicated by registration problems we encountered during MRI analyses that were also performed in our project. Of course a reference is in place here and has been added to the manuscript (reference no 21).

Ad 3) The 16 patients mentioned in the Methods were excluded during data analyses due to artefacts in the data. These 16 survivors were free from psychiatric disease or CNS disorders. Survivors with CNS disorders were already excluded in an earlier stage. Among those were 2 CRT treated patients with a meningioma discovered in the MRI part of our research. This information was added to the Subjects-section of the Methods. Two CRT-treated patients and 5 CT-treated patients who did not participate, were diagnosed with a psychiatric disease that we know of. But for most of the non-included survivors psychiatric status was unknown, so we do not know if these numbers are representative.
Ad 4) As requested, additional statistical results were added to the main text of the manuscript, regarding regional relative power differences, group differences on cognition, correlations with cognitive variables and the linear regression models.

Ad 5) Extra information was added to this subsection to clarify that the log transformation was sufficient.

Ad 6) Looking at correlations between regional theta powers and visuomotor accuracy of the preferred hand (Dr_pu), we see a consistent pattern of positive correlations across all regions (most significantly in the right central region), meaning higher theta power is associated with worse visuomotor accuracy. This contradicts an interpretation of increased theta being compensatory. But when the associations between theta power and visuomotor control are investigated controlling for the other regions (that is, all 10 regional powers plus age are entered into a linear regression model predicting visuomotor control and partial correlations are studied), beta’s are both positive and negative - the negative betas suggesting compensatory activity. But these negative beta’s do not reach statistical significance. The association within the right central region between increased theta power and decreased visuomotor control is the only significant finding.

Additionally, we looked for correlations between increased theta power and better cognitive performance on any of the assessed neuropsychological variables - so including those where the CRT patients did not differ from controls. We found a trend for an association between increased left occipital theta power and less attentional fluctuations and another trend for the association between left occipital theta power and better right hand visuomotor control on the tracking task.

Although it is just a trend of an association, adding the increase in left occipital theta power to the decrease in right occipital alpha2 power being associated with less attentional fluctuations (illustrated in the partial regression plots below), makes room for the possibility that attentional fluctuations are being counterbalanced by functional activity in the occipital lobes of the irradiated patients. These new results and discussion points were added to the manuscript.

Ad 7) Information was added to the Discussion that mortality or recurrence rates of CT treatment were similar or even better than the rates of CRT-treatment (reference n° 43).
Minor recommendations

Ad 1) Corrected.

Ad 2) Corrected.

Ad 3) The word “Additionally” was added to clarify this confusion.

Ad 4) Corrected.

Ad 5) Corrected.

Ad 6) In our opinion, the appearance of the bar chart with log transformed values is counterintuitive due to the negative values. To enhance interpretation and comparison of group means, line charts give a better representation, but with the downside of not being able to clearly display variability. Therefore, we added Supplementary Figure 1 with 4 graphs: graph A is a bar chart of the raw relative group means as submitted with the first version of the manuscript, graph B is a bar chart of the log transformed values, graph C is a line chart of raw values, graph D is a line chart of log transformed values. This way, we hope to provide full transparency to the reader, and demonstrate that the interpretations would not differ.

Ad 7) Corrected.

Discretionary recommendations

Ad 1) As suggested, this point got more elaboration in the Introduction.

Ad 2) A general recap was added to open the Discussion.

Regarding dr. Butz’s review:

Major compulsory revisions

Ad 1) It is widely accepted that the different α subbands reflect different cognitive processes. E.g. upper α desynchronization is selectively associated with the processing of sensory-semantic information, whereas desynchronization in the lower α band reflects attentional processes (Klimesch W: Memory processes, brain oscillations and EEG synchronization. Int J Psychophysiol 1996, 24:61–100 and Klimesch W, Freunberger R, Sauseng P, Gruber W: A short review of slow phase synchronization and memory: evidence for control processes in different memory systems? Brain Res 2008, Oct 15;1235:31-44). To the best of our knowledge, there is no reason for the other frequency bands to be subdivided.

Also, in many studies comparing ongoing EEG or MEG between patient groups and controls, differential effects have been seen in the upper and lower alpha bands. Changes in oscillatory power or changes in (graph-theoretical) network characteristics of the ongoing oscillations can be quite
different for lower and upper alpha bands. In fact, the same observation can be made in the present study.

Ad 2) We agree that correlations instead of causality were studied and adjusted the text accordingly.

There was a rationale for using age only as a covariate when theta was involved, namely that within the healthy controls a correlation between theta power and age could be established, but age-dependence was not an issue for controls in any of the other bands. Why throw out a degree of freedom when unnecessary? Despite that rationale, we can understand the argument of consistently using age as a covariate in the entire analysis. Therefore, we adjusted all analyses of variance, correlation analyses and linear regression computations using age as covariate while leaving out sample rate (see also point 3 of the minor essential revisions). This resulted in minor differences in outcomes.

Ad 3) We assume Dr. Butz would like us to correct for multiple comparisons in order to lower the chances of false discovery of significant results (the type I error rate). Traditionally, this would be pursued by applying the Bonferroni method. In a publication by Perneger (1998), a view widely held by epidemiologists is expressed that this method creates more problems than it solves and the best way of dealing with multiple comparisons is simply describing what tests of significance have been performed, and why (Pergener T: What’s wrong with Bonferroni adjustments. BMJ 1998, 316:1236-1238). We agree with Perneger, because this way, findings are not interpreted differently according to how many other tests were performed. Also, type I errors cannot be prevented without increasing the number of type II errors, which are equally worth avoiding.

Pre-established hypotheses were tested in our correlation analyses, instead of just correlating everything, and more than 50% of these hypotheses were confirmed. In addition, the correlation results were supported by the regression results. This, combined with reporting effect sizes in addition to significance levels, should provide the reader with sufficient information to base an interpretation of the results on.

Ad 4) The Discussion has been majorly revised and extended, also addressing major recommendation no 6 from Dr. Kleen.

Regarding the point of finding changes only in a minority of frequency bands, we would like to point out that these patients are mainly normally functioning adults, not diagnosed with any neurological disease that would have been reflected in other frequency bands. Subtle deviations were expected, mainly associated with cognitive deficiencies.

Minor essential revisions

Ad 1) Information was added to the Methods that patients were recorded for 10 minutes.

Ad 2) Adjusted.

Ad 3) We cannot think of any effect the sample frequency might have either. So, we are happy to leave it out as a covariate. But we see no reason to downsample the data. The same things were measured, just half of the data were measured in a more accurate way. For higher frequency bands,
we could see the relevance, knowing that a more accurate differentiation can be made between higher frequency bands using a higher sample rate. But this aspect is not relevant to our dataset.

Ad 4) This notion occurred to us just looking at the head plots of regional differences. We left out this sentence and just reported that there were no significant differences between the hemispheres.

Also, the concern about the sentence in the abstract has been addressed. The sentence was formulated more precise.

**Discretionary revisions**

Ad 1) The title was shortened.

Ad 2) In physics, the term *magnetic field* is used for two different phenomena:
1. the H-field is sometimes called the magnetic field, sometimes the magnetic intensity and sometimes the magnetizing field;
2. the B-field is also called the magnetic field, and sometimes the magnetic induction field.

These are, of course, different (albeit related) phenomena.

As MEG records the magnetic fields that are caused by the changes in the electric fields in the brain, due to synchronous and aligned electric currents in the brain, we believe it is more precise to use the term magnetic induction.

Of course the recordings are made on the outside of the head, and not from within the brain.

Ad 3) The Methods now state that the data have been log transformed, while the Results only report on the efficacy of this transformation.

Ad 4) Asterisks have been added to mark significant outcomes.

Ad 5) Figure legends have been adjusted.

We are grateful to the reviewers for their willingness to review our manuscript a second time and look forward to receiving their reviews. Hopefully, their concerns have been sufficiently eased.

Sincerely,

Marita Daams & Ilse Schuitema