Author's response to reviews

Title: Extreme sensitivity of gene expression in human SH-SY5Y neurocytes to ultra-low doses of Gelsemium sempervirens

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Author's response to reviews: see over
Object: MS: 8021020051005359 "Extreme sensitivity of gene expression in human SH-SY5Y neurocytes to ultra-low doses of Gelsemium sempervirens" Marta Marzotto, Debora Olioso, Maurizio Brizzi, Paola Tononi, Mirco Cristofoletti, Paolo Bellavite

Thank you for consideration of our revised manuscript for publication in your journal. We have improved the above manuscript according to your comments.

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Editorial comments:
- We feel that your manuscript would benefit from some discussion being included on possible confounding factors that might explain the effects that you see at high dilutions, other than those based in homeopathic principles. For example, these could relate to statistical limitations, or experimental artefacts. We feel that this would bring more balance to your Discussion section, and to your manuscript as a whole.

R: We welcome the Editor’s suggestion. We carefully considered all the possible artifacts and limitations, both in biotechnological issues and statistical approaches. Actually, the experimental setting was carefully designed as detailed in methods. However, thanks to this suggestion we took the opportunity to clarify some possible critical points to the readers and we have included a new paragraph in the Discussion section of the revised manuscript (p 21-22):

"Technical issues and confounding factors.

The puzzling evidence of gene expression changes under the influence of homeopathic dilutions prompt an analysis of the possible confounding factors that might explain the effects observed. We adopted different measures to address the issue of possible experimental artifacts. To avoid dye-bias artifacts a single-channel microarray was employed. We adopted a microarray design with probes of the same probe-set located in not contiguous positions on the array, so that artifacts due to uneven hybridization would only affect a subset of probes for a probe-set. Anyhow, the absence of spatial biases in fluorescence signal was assessed by checking the coefficient of variation of the mean signal intensities of different portions of each array. The experimental set up could have introduced biases and “position effects” if handling of control and Gelsemium s. matched dilutions was not equivalent. Actually, we conducted four independent experiments in which Gelsemium s. dilutions and the corresponding vehicle controls were processed in tandem (from drug addition to RNA extraction and cDNA synthesis). In every subarray of the chip, each transcript was targeted with three separate probes, merging the fluorescence values and attributing a statistical score.

Regarding the statistical analysis, the large number of genes of the complete set causes some problems concerning the choice of “interesting” genes. The approach followed here was quite stringent and limited the number of genes considered, reducing the probability of “false positive” results, but forcing to discard some possibly interesting genes from the analysis. Moreover, the small entity of the expression changes observed with high dilutions unavoidably reduced statistical inference in the single genes, especially since multiplicity corrections were applied. The choice of
analyzing the sign of the fold changes in a pool of genes, rather than the variance of a single gene, may lead to a loss of statistical information, to the advantage of greater precision in discarding the null hypothesis. Further research specifically oriented on the most responsive genes, with suitable sample sizes, could possibly overcome this limitation of the microarray approach.”