January 20, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15450-review.doc).

Title: DNA microarray unravels rapid changes in transcriptome of MK-801 treated rat brain

Author: Yuka Kobayashi, Sofya P Kulikova, Junko Shibato, Randeep Rakwal, Hiroyuki Satoh, Didier Pinault, and Yoshinori Masuo

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 15450

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer ***01172504***

(1) **Comments To Authors:** The article from Yuka Kobayashi et al., investigates the impact of Dizocilpine (MK-801) acute treatment on gene expression profile of different rat brain regions by microarray analysis. As suggested by authors, this analysis could help to identify potential biomarkers associated with neurological damage and sounds interesting for the readers of WJBC.
ANSWER: Thank you for the positive consideration of our manuscript and the comments below. These have helped us improve our manuscript, thank you very much.

(2) General comments:
A weakness of this work concerns the validation of microarray analysis. Only 2 genes (Cyr61, up-regulated, and Amy1 mainly down-regulated in all the brain regions) have been verified by RT-PCR. In my opinion the validation by qRT-PCR of the genes discussed in the Result session would significantly improve the work.
ANSWER: Yes, we agree, it would have been much better to have additional validation of the differentially expressed genes, but regretfully, at this stage where we do not have any more samples, tissues, total RNA, and cDNA due to their loss during the 2011 East Japan Earthquake and subsequent electrical shutdown (please see Number (6) below-Additional comments from the authors) we are not able to perform any additional experiments.

(3) Specific points Line 18 p13 (Table 2): I did not find a Table 2 in the manuscript received.
ANSWER: We apologize this should have been Table 1; where the gene-specific primers are listed. It has been corrected in the revised TEXT.

(4) Klf4 p16: Klf4 is up-regulated in several regions after MK-801 treatment. This seems to be in contrast with observations from other laboratory. The authors should better clarify this point.

ANSWER: Thank you for pointing this out; we have discussed this point in the revised text, and discuss further on the Zhu et al., 2009 paper, including citing four new references and discussions therein.

(5) Last line p17: remove "resulting" Figure 5: this figure shows data from a single experiment. The authors should indicate the number of experiments done and the standard deviation.

ANSWER: The offending word has been deleted form this sentence in the revised TEXT; in the section (*-Total RNA extraction, cDNA synthesis, and reverse transcription-polymerase chain reaction (RT-PCR)*), we have added new sentence indicating the analysis performed. Further as also mentioned in an comment to the other expert referee, we always confirm the best cycle in the PCR regime to measure the expression level at the most appropriate condition (not at the plateau).

(6) *Additional comment from the authors:* We would like to mention one point regarding the experiment and its samples; it was very unfortunate that I (including our group under Dr. Masuo at AIST, Tsukuba) lost all our samples (life-long works for 16 years) in the 3/11 Great East Japan Earthquake, and the following blackout for 1 week at the institute in Tsukuba, where I had been storing all my/our samples were being stored in a -80 degrees C deep freezer. So, I am unable to do any further experiments for this study, expect that in future we hope to do detailed bioinformatics analysis on the data that we have with us for these brain regions. As these data are massive, it will take time to analyze them in due time. This is not an excuse but the reality for us after the disaster in the year 2011.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry.*

Sincerely yours,

Randeep RAKWAL, PhD
Organization for Educational Initiatives,
University of Tsukuba,
1-1-1 Tennodai, Tsukuba 305-8577,
Ibaraki, Japan
E-mail: plantproteomics@gmail.com

January 20, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15450-review.doc).

Title: DNA microarray unravels rapid changes in transcriptome of MK-801 treated rat brain

Author: Yuka Kobayashi, Sofya P Kulikova, Junko Shibato, Randeep Rakwal, Hiroyuki Satoh, Didier Pinault, and Yoshinori Masuo

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 15450

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer ***01905258***

(1) **Comments To Authors:** The Ms. by Kobayashi et al. investigates the effect of MK-801 on gene expression in different areas of the rat brain. This represents an addendum to a previous study by Marvanová et al (Neuropsychopharmacology 2004; 29: 1070-1079) that identified genes regulated by MK-801 in the adult rat brain by cDNA microarrays analysis. This paper should be cited, and the published results by Marvanová should be compared with those obtained by the authors.

(2) **ANSWER: We appreciate the positive consideration of our manuscript and the critical comments below. These have helped us improve our manuscript, thank you very much. We apologize for overlooking this first citation from 2004. In the revised text, in the introduction, last paragraph we make a first reference to this paper and mention the differences amongst our and this study, as an introduction. Moreover, our search of the genes identified in the 2004 study with our gene lists from all the six brain regions identified only two genes in common; *Fos* (C-fos in Table 2 of that paper) and *Nfkbia* (RL/IF-1 in Table 2 of that paper). Interestingly, these two genes were found to be up-regulated in our study, and that to only in the High-dose experiment (we used 0.15 mg/kg MK-801); which makes it similar to the results obtained in the 2004 paper under high dose (1 mg/kg MK-801), a much higher dose than in our study. Therefore, we discuss these two genes in the revised text; results and discussion part (*Genome-wide transcriptome analysis reveals numerous and early changes in gene expression*). Another interesting point in common was that the gene expressions up-expression ratios were just above the 1.5 fold-change cut off value in both the analysis.**

(3) Other comments:

1. Fig. 3 has not been included in the current version of the Ms.

ANSWER: We apologize for this error; during pdf construction the figure was somehow not incorporated. Actually, the Editorial office had asked us to revise it before review, and we had corrected this error. Nevertheless, we are sorry that the referee was not able to see this figure during review. The revised text has this Figure 3.

(4) 2. Page 12, first sentence of last paragraph: What is the meaning of "> / < 1.5 / 0.75 fold"?

ANSWER: This indicates the fold-change value greater than or less than 1.5 and 0.75 fold-change in both chips in a dye-swap experiment. The offending sentence has been revised, and a new second sentence has been added after that (in section heading - *Genome-wide transcriptome analysis reveals numerous and early changes in gene expression*) on Page 13.

(5) 3. The RT-PCR analysis shown in Fig. 5 should include a gene of reference (beta-actin, GAPDH, or similar). In the absence of a reference no conclusions can be raised from this analysis (which should have been performed by real time PCR instead of classical RT-PCR).

ANSWER: Yes, you are right, but at this stage (please see Number (6) below-Additional comments from the authors) we are not able to do another experiment. However, in the Legend to Figure 5, we refer to the new statement - For the stable expression levels of *GAPDH* and β-actin genes, please see Figure 3 (B). On your comment to have performed the Real-Time / qPCR analysis, we respectfully would like to state that we have been utilizing the traditional PCR analysis in all our experiments since the past 20 years, with a particular reason in mind, and it does not anyway downplay the importance of qPCR analysis, which we feel is more suited to a single or few genes with tens or hundreds of different conditions. Although, the reviewer is an expert in this field, we would like to state our reason for using the old RT-PCR technique- *"Traditional RT-PCR is a long-standing and extremely powerful gene expression analysis tool, and is actually quantitative in nature. The Ethidium bromide dye is and has been used for the accurate quantification of DNA/RNA (Bonasera et al., 2007, BioTechniques), and it is only because it is considered toxic that other dyes have been developed. Traditional PCR detection involves use of various cycles by the researcher to measure the expression level at the most appropriate condition (not at plateau). We have done this undertaking various PCR experiments at various cycles and using gene-specific primers, making ours a quantitative approach. Following which the UV-transilluminator used a software/image analysis tool for quantifying the area/intensity of the bands and obtaining quantified values. Thus, in real terms, this method represents the quantitative values, is our view."* Finally, the text in the methods has been revised to express the experiment we did to confirm the reproducibility of these PCR analyses.

(6) 4. The Discussion section should comment on the physiological conclusions derived from the GO analysis

ANSWER: Thank you for this comment, we provide some additional discussion in this regard; in particular with the genes *Fos*, *Nfkbia*, *Klf4*, *Zfp36*, and *Ttr*. The discussions were determined based on the information available in the literature and we also tried to look at factors that modulate NMDAR-related synaptic activities. However, very less information is available in this regards, at this point. This also means that the vast genomic data we have gathered will be a new resource for the scientific community to further investigate and analyze.

(7) *Additional comment from the authors:* We would like to mention one point regarding the experiment and its samples; it was very unfortunate that I (including our group under Dr. Masuo at AIST, Tsukuba) lost all our samples (life-long works for 16 years) in the 3/11 Great East Japan Earthquake, and the following blackout for 1 week at the institute in Tsukuba, where I had been storing all my/our samples were being stored in a -80 degrees C deep freezer. So, I am unable to do any further experiments for this study, expect that in future we hope to do detailed bioinformatics analysis on the data that we have with us for these brain regions. As these data are massive, it will take time to analyze them in due time. This is not an excuse but the reality for us after the disaster in the year 2011.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Sincerely yours,

Randeep RAKWAL, PhD
Organization for Educational Initiatives,
University of Tsukuba,
1-1-1 Tennodai, Tsukuba 305-8577,
Ibaraki, Japan
E-mail: plantproteomics@gmail.com