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Journal of Visualized Experiments

Editor-in-Chief

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Dear Dr. Zhao Chen,

please find enclosed the revised version of our manuscript "Generation of topically transgenic rats by *in utero* electroporation and *in vivo* bioluminescence screening" where we have integrated the comments from the reviewers, as far as it was possible. Please find below a detailed response-to-reviewers letter.

Thank you very much
Regards

Carsten Korth

Response to Reviewers
JOVE50146R3, 23.4.13

Reviewer #1:
Manuscript Summary:
This manuscript describes a very useful improvement of the standard in utero electroporation technique. By co-electroporating a plasmid that encodes luciferase, cells which have been successfully electroporated with the sequence of interest can be tracked in vivo (this feature is very important) by intraperitoneally injecting a luciferase substrate to the pups and performing a bioluminiscence scan. This provides a very useful tool that will find wide interest among researchers using in utero electroporation. Application of the technique is illustrated with a specific experiment using DISC1.

→ We thank reviewer for this positive review

Major Concerns:
None

Minor Concerns:
None

Additional Comments to Authors:
On section 2.8.1, "checkmark" instead of "ceckmark" (spelling typo)

→ This typo has been corrected on page 6.

Reviewer #2:
Manuscript Summary:
In this manuscript, the authors describe an exciting method for in vivo screening of in utero electroporation (IUE) efficiency and general location. This is a valuable contribution to the field, as performing downstream behavioral analyses following IUE can be quite expensive, and there is great value in knowing these variables prior to engaging in behavioral analyses.

Major Concerns:
1. The postmortem analysis of the single brain compared to the in vivo luciferase signal on the same brain is highly valuable. The same verification process should be performed for figure 5, showing that the cortical versus hippocampal localization of luciferase activity in vivo is confirmed at the dissected brain level and in sections.

→ We have performed the early experiment depicted in Figure 6 on principal grounds, to establish that there is a gross overlap between factual localization of IUE neurons visualized by GFP fluorescence and the amount of bioluminescence signal obtained by IVIS recording. However, when we noted that GFP was not useful for in vivo quantitation of IUE neurons (see Figure 6D, and first paragraph on page 15), we did not co-transfect GFP any longer. We agree that the proposed experiment is interesting but argue that neither does the limited time allowance for revising this manuscript permit including this additional experiment, nor is the experiment essential for the filmed visual protocol proposed in this paper.

2. It is important to describe your exact criteria for including an animal in this particular behavioral assay. For example, do you need to pass a certain threshold level of signal? If so, what is that threshold. Also, in the discussion it would be worth commenting more about subject selection based on imaging - for example, certainly the behavioral assay chosen may well determine the level and region of the brain that should be targeted.

→ A sentence concerning the selection of animals has been included in the Discussion section (Page 14).

On page 2, last sentence of the 3rd paragraph of the introduction, we have mentioned that the reason for choosing amphetamine challenge test was that in a similar experiment in mice, Niwa et al. (ref. 18) had used this behavioral test successfully. We have added a sentence to the Discussion on page 18 where we state that the match between the IUE region and the behavioral test is dependent on the scientific question.

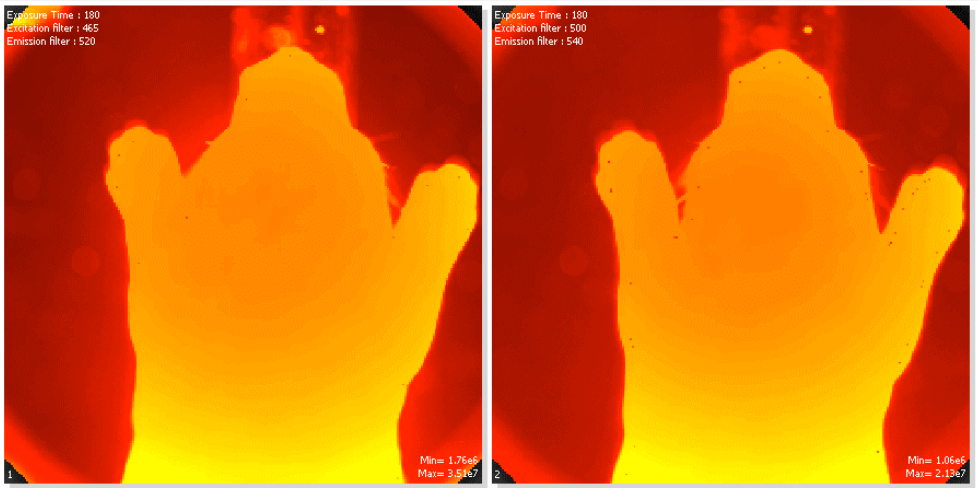
Minor Concerns:
1. The Figure numbering is all confused between the text and FIgures/figure legends. Also, Figures should be numbered based on their appearance in the text. Also, there is no image for Figure 1. Figure legend for Figure 6 has the wrong title.

→ Figure numbering in the text has been corrected throughout. The title of Figure 6 has been changed.

2. In the Intro, the authors mention that "...fluorescence signals could not be detected in postnatal stages in vivo...". Please add the detail of the method used for this. Same comment for "representative results" section.

→ Please see trouble shooting paragraph “GFP-fluorescence detection of the pups” on page 15 where details of the used methods have now been included.

Below an example of a test for fluorescence detection:
This is the same animal shown bioluminescence detection in Figure 6. For Fluorescence measurements of GFP two filter pairs (excitation/emission: 465/520 and 500/540) have been chosen, with an exposure time of 3 min.



3. In the abstract, I recommend highlighting the ability to longitudinally image these animals over time, which is quite valuable.

→ Due to the limited number of allowed words for the abstract, it is not possible to include more details about that. But the word "longitudinally over time" has been included (page 2).

4. please spell check the document

→ The entire document has been spell-checked.

5. It would be very valuable if the authors could comment in the discussion about the limit of detection for this system. Specifically, can they provide a rough estimate for the number of cells that are required to be hit in order to detect luciferase signal.

→ In the discussion on page 14 a few sentences about signal strength in the live imaging and counted GFP-labeled cells, has been included.

Additional Comments to Authors:
N/A

Reviewer #3:
Manuscript Summary:
In this paper Vomund et al examined the use of in vivo postnatal bioluminescent imaging technique to monitor the rat cerebral cortex and hippocampus, target regions where certain cells are overexpressed by luciferase expression plasmids via in utero electroporation during embryonic stages. The authors reported that postnatal intraperitonial injection of D-luciferin induces luciferase reaction, which is detectable by bioluminescence live imaging system at least up to postnatal day 35. The author also confirmed the feasibility of in utero electroporation to test long lasting effects of gene targeting on behaviors, by showing that overexpression of human DISC1 elicited an increase of amphetamine induced hyperlocomotion. Overall, monitoring in utero gene manipulation by in vivo postnatal imaging is very innovative and potentially useful, although there are some limitations which need to be addressed for publication.

Major Concerns:
Page 5, The author described that in utero electroporation at embryonic day 16 targeted the cells in layer II-IV of the cerebral cortex. However, gene targeting via in utero electroporation seems to be more layer-specific. For instance, in the case of mouse cerebral cortex, cells targeted at E14.5 are mostly differentiated at layers II/III.

→ This has been corrected on page 4 (section 1.3.5). Basically, we replaced the previous statement with the more neutral statement "upper cortical layers" quoting the paper by LoTurco et al. (ref. 23) where a similar statement was used accounting for the lack of unconsensus in this particular question.

Page12, 14, Niwa et al reported the behavioral effect of DISC1 silencing in the mouse bilateral prefrontal cortex, whereas the author tested overexpression of DISC1 in the unilateral cortical area. Is there any rationale to test unilateral, not bilateral manipulation?

→ Reasons and advantages to choose unilateral electroporation have been integrated in the discussion on page 14.

Figure 7, Although the data discerned from the bioluminescence image in Figure 6 suggest that sufficient number of cells are manipulated via in utero electroporation, the number of GFP-labeled cells in the Figure 7 looks very small. Please replace it with a more representative image to show if results are consistent.

→ We increased the quality of the picture so that the GFP-positive neurons are now better visible. The picture is very representative.

Tables in Page16, 28, Although the authors listed reagents of shRNA for Dab silencing in the tables, it seems to have no data showing the knockdown effect of Dab (reelin signal transducer?) via in utero electroporation in the text.

→ We apologize for the accidental insertion of "Dab1shRNA" vector information and have corrected for DISC1-overexpression vector information on page 16.

Minor Concerns:
Page3, the use of in utero electroporation for studies of neuropsychiatric disorders has been extensively discussed in the recent review article (Taniguchi et al, Neuroscientist 2012), which is better to be included as a reference.

→ The named Taniguchi et al. reference has been added in the introduction (ref. 19).

Page 16, catalog number of D-Amphetamine is missing.

→ catalog number for D-amphetamine was added on page 15

Tables in Page17, 28, 5mm of Tweezer electrode is CUY650P5, not CUY650P7. Please correct it.

→ Has been corrected on page 16.