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| |  |  | | --- | --- | | |  | | --- | | Dear reviewers,  We have addressed each of the points. Thank you for your input and positive comments regarding our manuscript.  Sincerely,  Daniel Vogt and John Rubenstein  **Editorial comments:**  1. References are missing DOIs. Please make sure that your references comply with JoVE instructions for authors. In-text formatting: corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text of the manuscript. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI, (YEAR).]  Response: We have used track changes to add the DOIs and the issue number to each reference. There is one reference, Batisto-Brito et al (reference #7), for which there is no issue number reported for, potentially based on the particular journal (Current topics in developmental biology).   2. Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.   3. Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.   4. If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.    **Reviewers' comments:**  **Reviewer #1:**  *Manuscript Summary:*  Cortical and hippocampal GABAergic interneurons are mainly derived from the embryonic medial and caudal ganglionic eminences (MGE and CGE); these interneurons have been implicated in psychiatric and neurological disorders including epilepsy, schizophrenia, bipolar disorders and autism. In this manuscript, using available Cre-driver lines and Cre-dependent expression vectors, Vogt and colleagues clearly developed a powerful technique that can efficiently transduce MGE stem/progenitor cells before transplantation.   Previously, people often inject Cre-reporter viruses into the brains of mice expressing Cre to observe the behaviors of restricted cells, however, viruses always do not spread far from the injection site. In contrast, MGE cells have the ability to migrate. Thus, the protocol described in this study can be used to investigate many interesting things, such as the behaviors of a specific subgroup of MGE cells that transduce with virus that expressing different genes.   That data are convincing, the images are high quality and the writing is clear. I only have some comments that need authors to answer in DISCUSSION.  *Major Concerns:* 1. Transplanting E13.5 MGE cells directly into P0-P3 cortex will bypass an important step in the development of interneurons-long distance tangential migration from the MGE to cortex. What is the by-effect of this method?  Response: The inability to assess tangential migration is a major drawback of transplantation directly into the neocortex. We have added text in the discussion, addressing how this particular aspect of cortical interneuron development can be addressed. This involves injecting transduced cells into the MGE of an age-matched embryo, thus allowing them to migrate and develop in the same manner as endogenous cells. For many labs, this is a hurdle because it requires major revisions and dedication to animal protocols, due to a more invasive procedure, while the transplantation into neonates is less invasive. However, we have added text to the discussion to address how to solve this dilemma if labs want to pursue these experiments.  *Minor Concerns:* 2. The authors should discuss the possibilities by performing transplantation experiments using CGE cells.  Response: This is a great idea. We have now added text in the discussion to talk about how one could also use Cre-driver lines to target CGE cells.   3. References 22, 23, 24, are missing in the manuscript.  Response: We only have 21 references in the manuscript. We have read over the text and any extra numbers have been addressed. We apologize for any confusion. This may have been confusing because we submitted a revised manuscript to be reviewed with track changes on, but the manuscript that had the changes had not been accepted. This may have led to the confusion and apologize for this. However, the version that we have now only contains 21 references, which have been verified to be present in the text.  **Reviewer #2:**  Vogt et al. describe powerful approach to target distinct subpopulations of interneurons in developing forebrain; specifically the neocortex. Interneurons are known to play significant roles in a number of neurodevelopmental disorders, including autism, schizophrenia and epilepsy. Therefore, advanced approaches to trace and manipulate distinct molecular mechanisms of interneurons, as described in this manuscript, are crucial for the advancement of the field. Authors not only report how to label different subpopulations, but also provide insight on how to manipulate molecules of interest at distinct time points of interest and in distinct subpopulations using an elegant combination of Cre-dependent expression lentiviruses and available Cre-driver mouse lines. In addition, described method has several advantages over other similar methodologies and is overcoming previous limitations in the field. Indeed, this is nicely written methods article by one of the leading groups at the forefront of brain development that constantly uses state-of-the-art advanced experimental methodologies.  Here are some minor suggestions that may contribute to the manuscript: A) Under 1.1 list what temperature and CO2 concentration was used to grow HEK cells.  Response: We have modified the text to list the temperature and CO2 content used to grow the HEK293T cells.  B) Under 4.4 - define into what volume of DMEM/10% FBS is MGE collected.  Response: We have modified the text to include a volume that we use that works well for this step of the protocol.   C) Under 5.2. authors could suggest with what to triturate the MGE tissue (pippette, 1 ml tip, or?)  Response: We have added text to this section that recommends using a P1000 pipette tip, which is what we have had success using.  D) Was CAG promter excised from pCAGGs using SpeI and XbaI sites ligated into SPeI and Xba1 sites of pLenti-CAG-Flex-GFP or only into XbaI site (page 9)?  Response: Thank you for bringing this clarity issue to our attention. Our insert had a 5' SpeI and a 3' XbaI restriction site and we ligated this fragment into the single XbaI site of the vector. This resulted in an insert which destroyed the 5' XbaI site yet conserved the 3' XbaI site. We have now added text in this section to make it more clear.   E) In Figure 5, authors could define in the figure legend what DPT stands for.  Response: We have added text to the figure legend to define DPT.   **Reviewer #3:**  In this protocol Vogt et al., describe a detailed procedure for lentiviral labeling of MGE cells as a method to genetically manipulate progenitors fated to become cortical GABAergic interneurons prior to transplantation. The research group has pioneered this protocol and approach and are authorities on its applications. Overall the manuscript is very well written, clearly outlining every step involved in the procedure with additional/ alternative strategies. I only have a few minor suggestions:   1) Page 2, Line 70-85: Since there are multiple publications on the same procedure, it may be useful to add a few sentences describing representative results and/ or advantages of this approach in the long abstract.  Response: We agree and have added another sentence at the end of the long abstract that states why this approach is advantageous (i.e. it combines powerful genetic tools and the ability of these cells to disperse from an injection site).  2) Page 3, Lines 127-128: Primary goal of the procedure is not to create injury but to inject MGE cells. I would rephrase 'site of injury' to 'site of injection'.  Response: We have deleted text that refers to this site as an injury and just refer to it as the injection site.  3) Page 7, Line 277: Please include trituration procedures and be consistent throughout the manuscript mentioning the pipettes used for trituration procedures. For example, section 6.3 (Page 8, Line 330) mentions P2 or P10 pipette for those steps but nowhere else.  Response: We have changed the text in 6.3 to focus on an optimal pipette. We also added text to section 5.2 in the trituration of the MGE cells as this was also mentioned by reviewer 2.  4) Page 7, Line 287: How long are MGE tissue incubated with the lentivirus? Incubation time is not mentioned anywhere except in the discussion (Page 12, Line 521).  Response: Thank you for noticing this lack in the protocol. We have now added text in section 5.3 to address the length of incubation time and a warning to incubation times that go to long.  5) Page 7, Line 319-320: with a clean paper towel.  Response: This was a difficult section, because JoVE policies do not allow us to state the actual protocol of using distinct products to remove excess media (i.e. Kimwipes), which is necessary to concentrate the pellet which will be injected. We have modified the text in section 6.3 to be more general and added an extra line about why this particular part of the procedure is important.  6) Page 9, Line 371: For readers who are not familiar with anesthetizing P1 pups by hypothermia, it would be useful to include a sentence or two on how to facilitate recovery from hypothermia.  Response: We have added text in the note below section 6.4 to address how one can recover a pup from hypothermia. | | |
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