The authors would like to thank the Editors and Reviewers for their comments on our manuscript. Below, we have included a list of changes made to the manuscript in response to comments that we have received. All line references mentioned in this summary correspond to the corrected and newly submitted manuscript (when tracked changes are not visible).

**Editorial comments:**

1. All of your previous revisions have been incorporated into the most recent version of the manuscript. In addition, Editor may have made minor copy edits to your manuscript and formatting changes to comply with the JoVE format. Please maintain these changes. On the JoVE submission site, you can find the updated manuscript under "file inventory" and download the microsoft word document.  Please use this updated version for any future revisions and track all changes using the track changes function in Microsoft Word.

* We used the manuscript mentioned above for our edits. Due to copy edits and formatting on the “Protocol” section, the numbering for lines 98 and lines 180 to 189 could not be corrected by the authors when steps were removed or added to the protocol.

1. Editor made formatting changes (line spacing), please maintain these changes moving forward.

* Changes to line spacing were maintained.

1. Formatting:
   1. The protocol should be formatted into Sections separated by section headings. Ethics approval should not be an experimental step. Only an ethics statement at the beginning of the Protocol section (below the "Protocol" sub-heading) is required.
      * We added a separate statement regarding ethics approval to the manuscript as the opening sentence in the Protocol (lines 83 and 84). When this change was made, subsequent numbering of the protocol section was changed (see earlier statement regarding the several protocol steps in which this wasn’t the case).
   2. Please express centrifuge speeds as “x g” rather than rpm (see 2.11.2).
      * Line 118 now states “Centrifuge blood at 1.5 x g for 25 minutes.”
2. Paraformaldehyde is toxic and requires a caution statement.

* We included the following statement on lines 177 to 179: “CAUTION: 4 % paraformaldehyde in PBS is carcinogenic and may also cause skin irritation, allergic skin reaction, or eye damage. Use appropriate eye/skin protection.”

1. Please include spaces between all numbers and units.

* Thank you, this has been corrected.

1. Please remove references to the video.

* The reference to the video has been removed.

1. Grammar:
   1. Title should be “Wheel running and environmental complexity as a therapeutic intervention in an animal model of FASD”

* The title has been corrected.
  1. Line 121 – “make them easier to identify animals”
* Line 129 (previously line 121) has been changed to “…make it easier to identify animals…”
  1. 5.1.2.3 – “Place lots” should be “Place a lot”
* Line 155 has been changed as recommended.
  1. Line 253 – “is introduce a”
* Line 290 (previously line 253) has been changed to “…is to introduce a…”
  1. Line 271 – “individual housing is widely accepted as detrimental to animals can even be directly detrimental”
* Lines 309 and 310 have been changed to “…individual housing is widely accepted as detrimental to animals and can even directly counteract the beneficial effects of…”

1. Additional detail is required:
   1. 2.7, 2.8 – How is intubation performed? This should be described or a citation should be provided.

* A citation was added on line 108 (Kelly & Lawrence, 2008) which provides a detailed protocol for the dosing procedure.
  1. 2.11.1 – Please describe how blood is collected or provide a citation.
* A citation was added on line 115 (Helfer et al., 2009) which describes the process of blood collection in its methods.
  1. Results:
     1. Please define the error bars (SD, SEM, etc.) in the figure legends. Please also define any statistical differences and indicate the statistical test used.
  + The figure captions were revised to include descriptions of error bars, statistical tests performed, and statistical results.
    1. Figure 3 – What treatments were used in A and B?
  + The postnatal treatment on animals used in the fluorescent photomicrographs in Figure 3 was AE (alcohol exposure), based on the laboratory records. Figure 3 was reproduced from a publication, and the initial publication does not include any details regarding the animal(s) displayed in the figure.

1. Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammatical errors. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

* The manuscript was proofread by the authors.

1. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

* The appropriate permissions have been uploaded to the Editorial Manager site in the appropriate section. The figure captions have been revised to include the statement, “This figure has been reproduced from [citation].” The also statement includes a superscript citation to the appropriate reference.

**Reviewers' comments:**

1. Reviewer #1:
   1. Minor Concerns:
      1. One suggestion is related to the role of the type of exercise. Currently she studies on exercise and its effects on brain plasticity show that the type of exercise and the duration of the program must be taken into account. I think it should include some mention of these topics of interest (see Ryan, S and Kelly(2016) Ageing Research Reviews, for example).
      * This was briefly addressed on lines 273 to 278. Further discussion and a citation discussing the implementation of these factors into rodent models of disease has been added on lines 278 to 280.
      1. On the other hand I consider relevant to mention the statistical analysis used in the different phases of study.
      * This has been added to the figure captions in the “Representative Results” section.
   2. Additional Comments to Authors:  
      it should also include some studies that show the different therapeutic effects of both interventions. So appears to be EC could have more influence on the anxiolytic responses while the voluntary exercise appears to modulate responses more related to cognitive functions (Rogers, J., et al 2016, Transl Psychiatry; doi:. 10.1038 / tp2016.52)
   * Discussion was added on lines 334 to 336 adding promise to the use of this paradigm in other models of rodent behavioral and neuroanatomical deficits.
2. Reviewer #2:
   1. Major Concerns:
      1. The major flaw of this protocol is that there is a lack of cell survival and neurogenesis effects by WR/EC in the control group. The pro-survival and neurogenic effects of enrichment are well documented and reproducible by independent labs. Therefore, the absence of that observation here indicates a major flaw to the proposed protocol. This has to be discussed as a major limitation to their approach, regardless of the impressive rescuing effects observed in the alcohol-exposed rats.
      * The neurogenic effects of “enrichment” in the field are dependent primarily on the inclusion of a running wheel or some alternative form of cardiovascular exercise in the environment (e.g., references 9,51 ). The purpose of this manuscript is to propose an intervention that has been demonstrated in the literature and allows for the contribution from each of the two elements to be independently identified. Additionally, this protocol proposes the use of this “superintervention” as a therapeutic to rescue alcohol-induced deficit. For this reason, the authors respectfully disagree with reviewer #2’s comments above as major limitations to the proposed protocol.
      1. I suspect it may be due to the lack of detail in describing Section 5. Environmental Complexity. There is only mention of different sized toys, but these could be solid toys rather than objects which provide the rats housing opportunities. There is also no mention of nesting material (e.g. shredded paper, straw) to promote nest building and digging behaviour, tunnels to promote exploratory behaviour.
      * The items in the environmental complexity paradigm should be highly varied (as mentioned on line 150). Since there should be a variety of different objects that should not be consistent, the authors decided that it would be too cumbersome to list the wide variety of objects used, and believed that individuals interested in the specific items would be able to see the items in the accompanying video protocol. Novel nesting material is not used in this paradigm, and a statement has been added on line 148 that states that the cage should include standard bedding. Among the various items used in this protocol, some are tunnel-like, but the authors’ protocol does not mandate a certain number of tunnel-like objects. Animals in all treatment groups explore the objects used in this paradigm (see reference 49) and thus it is unnecessary to state that tunnels would be required to promote exploratory behavior.
      1. The constant configuration changes (every 2 days) and cage cleaning (3 days), but especially the latter, is stressful to rodents. I cannot help wonder if the absence of a significant up-regulation of neurogenesis reflects this since stress is well-established to reduce neurogenesis.
      * The citation proposed by Reviewer #1 (Rogers et al., 2016; reference number 53) added in response to a comment by Reviewer #1 (comment 1.b., above) discusses anxiety in response to an EC paradigm. Specifically, this reference addresses that EC reduces anxiety-like behavior. Further, we could not determine an empirical basis that would implicate any stressful effects of cage cleaning as responsible for the lack of survival in adult-born hippocampal granule cells in suckle control animals (but not sham-intubated or alcohol exposed animals).
   2. Minor Concerns:
      1. The authors should substantiate the dosage indicated in point 2.5, 2.5.1. Are there references for these doses?
      * A citation was added to line 105 to indicate the model from which this dose is derived.
      1. 2.11.1 Is blood collected in an EDTA tube for plasma or serum? Authors should mention that blood sampling 1.5hr after intubation gives the maximal blood alcohol readout.
      * An additional protocol step was added (1.11.4.) to address that the blood collected provides serum for further analyses. Additionally, a parenthetical statement was added on lines 113 and 114 addressing the reason for collecting blood at 1.5 hours after exposure.
      1. The stringent need for litter sizes to be 8, makes the flexibility for weaning numbers (2-3) odd. Especially since 3.1.2. suggests at least 3 rats per cage. Surely a minimum of 3 would be a preferable approach.
      * This is explicitly addressed in section “2. Weaning” (previously, “3. Weaning”). Line 134 states that each cage only contains one animal from each condition. Line 135 further describes that the 3 animals in a single cage should not be from the same litter if possible, thus the use of 2-3 animals per cage is not unusual. To address the possible need for an experiment to use different litter sizes, step 1.1.1. was added to discuss the considerations that led to the decision of 8 animals per litter.
      1. More detail might be required for 4.1. WR description. I believe there are commercially available rat cages with wheels that automatically log the usage.
      * The “Instructions for Authors” document says to avoid the use of commercial language or company brand names in the document. Additionally, the authors included the wheel that their lab uses which is not similar to the item suggested by Reviewer #2 and thus it was not included in the manuscript or materials list. It is also unclear in what way a wheel would “log usage” that the model listed by the authors (in their materials list) does not, as all animals in the cage will have an access to the wheel and no individual recording is possible.
      1. There should be an additional point 6.6 to conclude the section of tissue collection. 'Fixed brains are cryopreserved or paraffin embedded in accordance to teh requirements of the subsequent sectioning and immunohistochemical analyses.'
      * The reference given in the note below step 5 (line 177) includes a detailed description of the procedures following the tissue collection protocol explained in this manuscript. Although this manuscript describes perfusion with paraformaldehyde, the authors had previously included the note that, “Tissue collection can be performed with a variety of methods (e.g., perfusion with paraformaldehyde, rapid decapitation, etc.).” This note was revised to include flexibility in tissue storage techniques as well.
      1. Asterisks in Fig 1C, 2C, and 3C are misaligned.
      * Figures 1, 2, and 3 are reproduced from a previously published article. The authors reproduced these figures and did not alter them in any way.
      1. The table needs to be aligned properly. Also the description of small, medium and large objects is poorly included, as it provides no information to the reader.
      * The cells in the excel spreadsheet materials table have been aligned to the top left corner. Details regarding the specific objects are intentionally left vague as a wide variety of objects are used in the environmental complexity cage, and quantification of each of these objects (and the specificity of the brand and item) would make the list incredibly cumbersome. The authors decided (as addressed above) that the accompanying video protocol would provide an appropriate supplement to the list and manuscript.