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| **Author Checklist** | Manuscript Number: |
|  | NCOMMS-25-23244A |
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| **Please check the items below carefully and add a response in each row of the table to indicate the changes that you have made. Please also check through any additional marked-up edits we may have provided within the manuscript file.** |  |
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| **Abstract and editor's summary** |  |
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| We would like to propose a revised title to comply with our formatting requirements and improve the accessibility of your work:  Please take into account R2's comment about the title (paragraph 3)  Please edit the title in your manuscript files accordingly. Note that further minor changes may be made during the production process, and you will be able to check these in the proofs. | In accordance with the editor’s guidance and Reviewer #2’s comment regarding the emphasis on modularity, we have revised the title to:  **The ANTsX ecosystem for mapping the mouse brain**  which we believe accurately reflects the manuscript’s focus on reproducible, ANTsX-based workflows for mouse brain mapping. |
| Your paper will be accompanied by the following editor's summary. Please let us know if there are any inaccuracies: 'We request that you (the author) please supply a brief (~250 characters, including spaces) two-sentence summary of the main findings of the paper to be used on our website and in our e-alerts. The summary should be written in the third person in language suitable for a broad audience. The first sentence should describe an open question (i.e., the rationale/motivation for the present study), and the second sentence should describe what you found/demonstrated in your manuscript. The summary may be edited by the editors prior to publication. Example #1: The neural mechanisms that give rise to aperiodic EEG signals remain unclear. Here the authors characterize EEG signals generated by neural processes other than brain rhythms, demonstrating that certain drugs alter EEG signals in ways that confound traditional interpretation. Example #2: Encoding and downsampling images is key for visual prostheses. Here, the authors show that an actor-model framework using the inherent computation of the retinal network yields better performance in downsampling images compared to learning-free methods. ' | We have provided the following two-sentence summary:  *Integrating molecular and anatomical data in the mouse brain requires flexible, reproducible mapping tools. Here the authors demonstrate ANTsX-based workflows for aligning MERFISH, fMOST, MRI, and LSFM data into shared coordinate frameworks.* |
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| We can accommodate up to 10 display items (Figures or Tables) in the main article. Each Figure and Table must fit easily within an A4 page (210 x 297 mm). Please ensure that the number and size of your Figures and Tables fulfil these requirements to avoid any delay in the acceptance of your article. | The main manuscript contains a total of ten display items (Figures and Tables combined), each formatted to fit within a single A4 page. Figure and Table dimensions have been checked to comply with Nature Communications layout requirements. |
| Ensure main Figures are uploaded as separate individual files. Each figure file must contain all intended panels labelled and displayed as intended and fit entirely on a single page. Do NOT include legends within the figure files, as these must be in the main manuscript.  Supplementary Figures must be all contained in the Supplementary Information PDF and do NOT need to be uploaded separately. | All main Figures have been uploaded as separate, individual files, each containing the complete set of labeled panels and formatted to fit within a single page. Figure legends are included only in the main manuscript. |
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| We do not edit Supplementary Information files; they will be uploaded with the published article as they are submitted with the final version of your manuscript. Any tracked changes should be removed from the file and the file should be provided as a PDF file. Supplementary Figures do not need to be provided separately. | No Supplementary Information files accompany this manuscript. All figures fully reproducible using the data and scripts provided in the public GitHub repository ([*https://github.com/ntustison/ANTsXMouseBrainMapping*](https://github.com/ntustison/ANTsXMouseBrainMapping)). |
| Please supply Source Data files for all data presented in graphs within the Figures. | An additional copy of all source data files for all data presented in graphs within the Figures are included with this submission. |
| Within the Source Data file, the relevant raw data from each figure or table (in the main manuscript and in the Supplementary Information) should be represented by a single sheet in an Excel document, or a single .txt file or other file type in a zipped folder. An example of the Source Data file is available demonstrating the correct format: https://www.nature.com/documents/ncomms-example-source-data.xlsx The file should be labelled 'Source Data', with the title and a brief description included in your response here, and should be mentioned in all relevant figure legends using the template text below: 'Source data are provided as a Source Data file.' A reference to the source data file should be added in the 'Data Availability' section, using the text: Source data are provided with this paper. | All data underlying the relevant figures are provided as .csv and .log (i.e., raw text) files within the public GitHub repository ([*https://github.com/ntustison/ANTsXMouseBrainMapping*](https://github.com/ntustison/ANTsXMouseBrainMapping)). |
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| **Main text** |  |
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| Please provide the source data used for generating figures as a single excel file (i.e., each figure should have its own separate sheet tab). If the manuscript includes data from both males and females, please disaggregate data points by sex in the source data file as detailed in the SAGER guidelines. | As specified previously and detailed in the manuscript, all the numerical data used to produce the relevant figures, as well as the scripts, are available in the GitHub repository associated with this manuscript. An additional copy of source data is included with this submission. |
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| If bold/italic formatting in tables is necessary, please define its meaning in a table footnote | Table 1 is free of bold/italic formatting. |
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| Please supply a brief (maximum 250 characters, including spaces) summary of the main findings of the paper to be used on our website and in our e-alerts. The summary should be written in the third person in language suitable for a broad audience. The summary may be edited by the editors prior to publication. Please provide this summary in your cover letter. | We propose the following two-sentence summary for use on the journal website and e-alerts: Mapping diverse mouse brain data into shared reference spaces is key for integrating structural, molecular, and developmental studies. The authors demonstrate how the ANTsX ecosystem enables reproducible alignment across these modalities.  (Need to provide the summary in the cover letter). |
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