

Spatial transcriptomic atlas of murine neurotoxocariasis reveals region-specific host responses and dysfunction in the brain

Corresponding Author: Dr Guangxu Ma

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This manuscript addresses the interaction of parasitic *Toxocara canis* larvae with the brain as well as with the immune system more broadly; the study takes novel approaches with spatial transcriptomics, single nucleus RNA sequencing, and immune modulation by parasite products, as well as alteration in parasite gene expression by infected host serum. However, the breadth of scope is not well matched by depth of analysis, in particular lacking control comparisons and, with few exceptions, making broad-sweep statements that lack specific examples.

After a well-written Introduction that sets out the context of neurotoxocariasis, the Results section covers spatial transcriptomics of infected brain tissues (Figures 1 and part of Figure 2), a serum lipidomics assay (also Figure 2), larval gene expression in vitro when exposed to host serum (Figure 3), the effects of certain parasite proteins on macrophage responses in vitro (Figure 4), and behavioural changes in infected mice (Figure 5). While the in vitro experiments in Figures 3 and 4 contains some interesting data, they do not relate to the main topic of the paper and would better be presented in a different manuscript.

Specific Criticisms

1. The study analyses the transcriptome of different regions of the brain in *T. canis* infected mice, but does not include uninfected mouse brain controls; hence it is difficult to speak of “responses” (lines 2, 239, 254 and elsewhere) because there is no baseline comparator. Without such control statements such as those in lines 112-115 are unsupportable. In Figure 2 there is a comparison between 2 foci containing parasite larvae, and 2 without: this is useful but given later data on systemic changes in infection, the larval-free tissues from infected mice are not an adequate control.
2. The authors speak of “lesions” that were observed macroscopically (line 72) but these are not imaged; “sites of parenchymal damage and haemorrhage” are reported (lines 98-99) with reference to Fig 1 e, but these are not evident or indicated in the Figure. Although the section is entitled “Localised immune recognition” (line 97) there are no data showing that, for example, microglia are accumulating at the lesion site, only that there are good numbers at the meninges and cortex, which is to be expected. (Incidentally the wording in lines 100-101 is confusing, is the “cell aggregation” of microglia or endothelial/mural cells?).
3. The cell composition data in lines 108-115 and Suppl. Figs 2 and 3 are not possible to interpret without comparison to uninfected brains; it is not adequate to reference a different study which may not use the same protocols or bioinformatic methods.
4. In line 127 the authors state that parasite transcripts were abundant in brain slices S4, S5 and S6; yet in lines 117-120 they report that microglia predominated only in S4, not S5 or S6. This is not consistent with the statement that “key antigen-presenting cells are in close vicinity to migrating *T. canis* larvae”, as these larvae are present in S5 and S6 as evidenced by presence of their transcripts.
5. Figure 2 contains lipidomic analyses that, like Figures 3 and 4, do not sit well with the spatial neurobiology theme; it should be omitted unless the authors also have data on key lipid changes in the infected brain.
6. I will limit comments on Figures 3 and 4, as mentioned above I think they should be moved to a separate publication. However, the morphological definition of M1 vs M2 (Suppl. Fig 4) is very weak and should be omitted. The molecular data (Figure 4 c-f) represents a better method, but shows important inconsistencies, for example suppression of IL-1b transcript

while enhancing protein levels, and sharp contrast between effects of 10 ug/mL and 15 ug/mL rTCPEBP1 in induction of PPAR γ and Arg1. There is no statement in the Figure Legend about independent replicate experiments for these data.

7. The treatment of larvae with serum from mice of different status is shown in Figure 3, but despite using pregnant infected mouse serum, the sex of the donor animals is not detailed, and pregnant uninfected serum is not tested.

8. Figure 5 sets out a spatial map of cell types in the infected brain. It is striking that different populations localise so differently, which counteracts the authors' statements of localised immune reactions – if so why don't the different cells co-localise? In any case it is not clear from the Figure where the lesions are, and for some reason different cell types are shown for the different slices.

9. Figure 5 also presents some useful data on behavioral alterations in infected mice, compared to uninfected controls. Why in Figure 5 f is there such a sharp change in activities between days 45 and 48, shouldn't these be at the same level?

10. The manuscript is replete with generalised claims and statements that have little meaning, for example the sentences on lines 94-95, 143-144, 155-156, 166-167, 169-170.

11. Many of the Supplementary Tables are disjointed and disorganised. The files appear to be PDFs of large spreadsheets in which adjacent pages have been separated so that it is impossible to read across the columns. There does not seem to be any coherent order to how entries are ordered, neither alphabetical or by magnitude of any particular value. This is an important issue as the Figures (eg Figure 2) do not detail any specific genes that are differentially expressed.

Reviewer #2

(Remarks to the Author)

This manuscript presents a highly ambitious and methodologically sophisticated investigation that integrates spatial transcriptomics, single-nucleus RNA sequencing (snRNA-seq), lipidomics, and behavioral assays to explore regional and molecular host–parasite interactions during *Toxocara canis* neuroinvasion in a murine model. The study offers valuable insights into the immunopathological, endocrine, and neurological dimensions of neurotoxocariasis, addressing a critical gap in our understanding of parasitic infections of the central nervous system. The findings are novel and supported by a substantial body of data.

Nonetheless, I believe the manuscript requires major revision before it is suitable for publication. Specifically, the authors should temper some of the mechanistic interpretations, enhance the translational framing, and more clearly articulate the limitations of their experimental models and behavioral assays. My detailed concerns are outlined below:

1. The manuscript draws strong conclusions linking *T. canis* larval presence to region-specific neuronal dysfunction and behavioral deficits. While spatial transcriptomic overlays and behavioral data suggest such associations, the evidence remains correlative. Establishing causality would require additional experimental controls—such as stereotactic injection of excretory-secretory (ES) products or immunological mimics—to disentangle the effects of larval activity from immune activation or systemic inflammation. Please clarify the limitations of inferring causality from co-localization and expand the discussion to include alternative interpretations of the observed phenotypes.
2. The transcriptomic data implicating Tc-PEBP-1 and dauer-like gene programs are compelling. However, the conclusions regarding immune modulation and endocrine reprogramming rely heavily on in vitro findings and predicted molecular interactions. These should be explicitly framed as hypotheses requiring further in vivo validation. Incorporating functional assays—such as antibody blockade or RNAi-mediated knockdown of Tc-PEBP-1—would substantially strengthen the mechanistic claims.
3. The rotarod and Morris Water Maze tests indicate mild-to-moderate impairments in motor learning and memory. However, small sample sizes and the absence of longitudinal tracking constrain the interpretability of these results. Additionally, the predator cue avoidance test yielded no significant differences, yet is discussed as corroborative. Please include power analyses for the behavioral outcomes, clarify the reliability and sensitivity of each assay, and moderate conclusions regarding neurocognitive deficits accordingly.
4. The manuscript situates the findings within a human health context, referencing disorders such as schizophrenia and Parkinson's disease. However, murine neurodevelopment, immune privilege, and glial architecture differ markedly from humans. The authors should more clearly position their findings as exploration within a model system and avoid direct extrapolation to human neuropathology without appropriate caveats regarding translational relevance and interspecies differences.
5. The manuscript notes that mouse sex was not controlled for. This should be acknowledged as a limitation, with reference to known sex-specific differences in CNS immune responses and neuroinflammatory outcomes.
6. The spatial transcriptomic data would benefit from improved clarity. Specifically, a more explicit distinction between host and parasite signals would aid the reader's interpretation and enhance the impact of visualizations.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have made considerable revisions to their manuscript in response to the Reviewers' critiques, including conducting new experiments and re-drafting multiple sections of the text. Although much improved there are some remaining points requiring attention:

1. A central point was the absence of uninfected brain tissue controls from both spatial transcriptomics (Figure 2) and RNAseq (Figure 3). The authors responded by conducting RNAseq on uninfected brain tissue, but only show a very brief (and almost unintelligible) comparison of pathways added on to Figure 3 with one very general sentence in the Results (lines 166-168). What do the colors in Figure 3 j-l represent? In presenting Figure 2 the authors' text should clarify that only infected mice were analyzed.
2. The text accompanying Figure 3 (lines 152-157) give detailed cellular analysis only on infected brain tissues and do not mention any comparisons with uninfected tissues. Here it is critical that the authors should give more prominence to the infected/uninfected comparison, extending Figure 3 j-l with specific gene levels as well as pathways, and adding similar analyses of imputed cell types, that would justify the statement on lines 170-171 about broader systemic effects of infection.
3. The authors need to double check their reference list. For example Ref 27 has no connection with studies on the fuzzy coat (lines 212-213), while in the Discussion (line 301) Ref 35 has no relation at all with the interaction of PEBP with macrophages.
4. Line 268-270 (Figure 6): there seems a contradiction here; infected mice spend less time in the predator-scented compartment, hence their fear-related behavior is accentuated not suppressed. The Figure panels and legends are poorly explained, so perhaps this is just a presentational error, but given the prominence of the authors' claim that neurotoxocariasis has behavioral sequelae, it is essential that this is fully documented.
5. Lines 460-462 : Why was it necessary to make a new NCBI database deposit for Tc-PEBP, (PV590067) when it already exists as U29761 from 1995, which is not acknowledged or the prior publication cited in the manuscript.

Reviewer #2

(Remarks to the Author)

The authors have addressed my major concerns appropriately; therefore, I recommend the manuscript for acceptance in this respected journal.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

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10 January 2026

RE: Manuscript NCOMMS-25-39223; Title: Spatial transcriptomic atlas of murine neurotoxocariasis reveals region-specific host responses and dysfunction in the brain

Many thanks for reviewing our manuscript, particularly for your detailed and constructive reports. In the following, please find a rejoinder that addresses your concerns and the reviewers' issues in a point-by-point manner (the authors' responses are in bold-type).

Reviewer #1 (Remarks to the Author):

This manuscript addresses the interaction of parasitic *Toxocara canis* larvae with the brain as well as with the immune system more broadly; the study takes novel approaches with spatial transcriptomics, single nucleus RNA sequencing, and immune modulation by parasite products, as well as alteration in parasite gene expression by infected host serum. However, the breadth of scope is not well matched by depth of analysis, in particular lacking control comparisons and, with few exceptions, making broad-sweep statements that lack specific examples.

After a well-written Introduction that sets out the context of neurotoxocariasis, the Results section covers spatial transcriptomics of infected brain tissues (Figures 1 and part of Figure 2), a serum lipidomics assay (also Figure 2), larval gene expression in vitro when exposed to host serum (Figure 3), the effects of certain parasite proteins on macrophage responses in vitro (Figure 4), and behavioural changes in infected mice (Figure 5). While the in vitro experiments in Figures 3 and 4 contains some interesting data, they do not relate to the main topic of the paper and would better be presented in a different manuscript.

RESPONSE: Thank you very much for your valuable, constructive comments. In response, we have undertaken a major revision that includes new experimental data, re-analysis of data with key controls, and a thorough refinement of the text to ensure that the conclusions are well-supported. The key changes are detailed below.

Specific Criticisms

1. The study analyses the transcriptome of different regions of the brain in *T. canis* infected mice, but does not include uninfected mouse brain controls; hence it is difficult to speak of “responses” (lines 2, 239, 254 and elsewhere) because there is no baseline comparator. Without such control statements such as those in lines 112-115 are unsupportable. In Figure 2 there is a comparison between 2 foci containing parasite larvae, and 2 without: this is useful but given later data on systemic changes in infection, the larval-free tissues from infected mice are not an adequate control.

RESPONSE: Many thanks for your critical comments. We agree that a baseline comparator is essential. We have now produced snRNA-seq data from uninfected control brains (n = 3). Comparative analysis between infected and uninfected brains is presented in the new Fig. 3 (and Supplementary Table 5). This has allowed

us to rigorously define infection-specific “responses” (e.g., lines 148-177). All statements regarding host responses throughout the manuscript have been verified and revised according to this new comparative baseline and the findings.

2. The authors speak of “lesions” that were observed macroscopically (line 72) but these are not imaged; “sites of parenchymal damage and haemorrhage” are reported (lines 98-99) with reference to Fig 1 e, but these are not evident or indicated in the Figure. Although the section is entitled “Localised immune recognition” (line 97) there are no data showing that, for example, microglia are accumulating at the lesion site, only that there are good numbers at the meninges and cortex, which is to be expected. (Incidentally the wording in lines 100-101 is confusing, is the “cell aggregation” of microglia or endothelial/mural cells?).

RESPONSE: Many thanks for your observation and criticisms. We have included a new panel (Supplementary Fig. 1a) with macroscopic images of brains from infected mice (n = 6) that were used in Stereo-seq, single-nucleus RNA-seq and lipidomic analyses, where parenchymal damage and haemorrhagic lesions are clearly visible. Lesions are not indicated in Fig. 1 (brain section images) due to the presentation of macroscopic findings. Migrating larvae are indicated in the brain section images in Supplementary Fig. 4 to provide more information.

Localised immune recognition is now described in the text (lines 97-100, 115-122, and 152-157). The data show that while microglia are present in meninges/cortex in slices with low parasite load (S1-S3), their annotation was notably absent from slices with high parasite transcript burden (S4-S6), suggesting a distinct, region-specific immune microenvironment. We have carefully revised the text to explain this.

3. The cell composition data in lines 108-115 and Suppl. Figs 2 and 3 are not possible to interpret without comparison to uninfected brains; it is not adequate to reference a different study which may not use the same protocols or bioinformatic methods.

RESPONSE: Many thanks for your critique. We have now included snRNA-seq representing uninfected controls for direct comparison (Fig. 3). We acknowledge that the spatial transcriptomics (Stereo-seq) dataset lacked an uninfected spatial control, which limited direct quantitative interpretation of cell abundance from that assay alone. Therefore, we have revised the text to be more cautious, stating that “despite the identification of molecular signatures indicative of antigen-presenting cells (e.g., macrophages and dendritic cells) and immune effector cells (e.g., B cells, T cells, granulocytes), the abundance of these cells was very low” (Lines 107-110), framing this as supportive evidence rather than a quantitative measure.

4. In line 127 the authors state that parasite transcripts were abundant in brain slices S4, S5 and S6; yet in lines 117-120 they report that microglia predominated only in S4, not S5 or S6. This is not consistent with the statement that “key antigen-presenting cells are in close vicinity to migrating *T. canis* larvae”, as these larvae are present in S5 and S6 as evidenced by presence of their transcripts.

RESPONSE: Thank you for your observation and criticism. We apologise for the lack of clarity. Compared with S1, S2, and S3, only small numbers of microglia were identified in slices S4, S5 and S6, with a relatively large number of microglia found surrounding migrating larva in the S4. The findings were supported by the snRNA-seq data for brain sections adjacent to *T. canis* larvae (invaded slices: S4a, S5a and S6a) and on

corresponding uninvaded control slices (S4c, S5c, S6c) from the same anatomical regions. We have carefully revised the text (lines 107-110, 135-136, and 156-157), to supporting the concept of a localised, rather than widespread, immune recognition.

5. Figure 2 contains lipidomic analyses that, like Figures 3 and 4, do not sit well with the spatial neurobiology theme; it should be omitted unless the authors also have data on key lipid changes in the infected brain.

RESPONSE: We very much appreciate your constructive critique and suggestions. We agree with your comment that key lipid abundance changes in the infected brains of mice are important, and necessary to interpret with the lipidomic changes in the sera of infected mice. We have conducted new lipidomic profiling of brain tissue from infected vs. uninfected mice (n = 6 per group). These new data (Fig. 4a-d, Supplementary Table 4) reveal significant lipid remodelling in the infected CNS, characterised by membrane degradation and oxidative stress signatures. We now link serum lipidomic changes to the pathological state in the brain.

6. I will limit comments on Figures 3 and 4, as mentioned above I think they should be moved to a separate publication. However, the morphological definition of M1 vs M2 (Suppl. Fig 4) is very weak and should be omitted. The molecular data (Figure 4 c-f) represents a better method, but shows important inconsistencies, for example suppression of IL-1b transcript while enhancing protein levels, and sharp contrast between effects of 10 ug/mL and 15 ug/mL rTCPEBP1 in induction of PPARg and Arg1. There is no statement in the Figure Legend about independent replicate experiments for these data.

RESPONSE: Thanks a lot for your comments and valuable suggestion. The *in vitro* macrophage stimulation data (original Fig. 4) have now been removed from the manuscript. The data on larval response to host serum (original Fig. 3) has been retained but included in Fig. 5 and the rationale clarified; the findings indicate a molecular dialogue where host systemic factors influence parasite stress adaptation pathways (e.g., dauer entry), relevant to understanding the parasite's survival/persistence in the brain.

7. The treatment of larvae with serum from mice of different status is shown in Figure 3, but despite using pregnant infected mouse serum, the sex of the donor animals is not detailed, and pregnant uninfected serum is not tested.

RESPONSE: Thanks for your comment. We have revised the manuscript to address these issues. We re-tested serum from pregnant uninfected mice and included these data in the analysis (Supplementary Table 6). All mice used in the study were female, and this information has been added to the *Methods* section (Line 360). The integration of data provides a complete experimental matrix (uninfected, infected, pregnant uninfected, pregnant infected) for analysis and an interpretation of hormonal effects on the parasite (Lines 378-389).

8. Figure 5 sets out a spatial map of cell types in the infected brain. It is striking that different populations localise so differently, which counteracts the authors' statements of localised immune reactions – if so why don't the different cells co-localise? In any case it is not clear from the Figure where the lesions are, and for some reason different cell types are shown for the different slices.

RESPONSE: Thank you for your appraisal. We have moved the spatial cell atlas to Supplementary Fig. 4 to avoid confusion with the main figures. The primary spatial analysis (Fig. 1) shows consistent cell types and distributions. The variability seen in Supplementary Fig. 4 likely relates to biological variation and to the

challenge of annotating rare cell populations from spatial data.

As mentioned in our response to Comment 2, lesions are not indicated in Fig. 1 due to the presentation of macroscopic findings. Apart from migrating larvae, lesions were not indicated in the original Fig. 5. For this reason, different cell types with asymmetrical distributions (indicating ‘lesions’) are shown in distinct slices. We have used circles to indicate to indicate migrating larvae in Supplementary Fig. 4 (described in the legend).

9. Figure 5 also presents some useful data on behavioral alterations in infected mice, compared to uninfected controls. Why in Figure 5 f is there such a sharp change in activities between days 45 and 48, shouldn't these be at the same level?

RESPONSE: Many thanks for your constructive comments, and for your observations. Learning occurs across trials. We have repeated the Morris Water Maze experiment with an expanded cohort (n = 12 per group) over days 55-60 post-infection. The results are consistent (see revised Fig. 6): infected mice show a significantly slower rate of improvement (longer escape latencies) and impaired memory recall, confirming a deficit in spatial learning over time, not just on a single day.

10. The manuscript is replete with generalised claims and statements that have little meaning, for example the sentences on lines 94-95, 143-144, 155-156, 166-167, 169-170.

RESPONSE: We are grateful for your critique. We have revised the wording/statement and make it more accurate and meaningful.

11. Many of the Supplementary Tables are disjointed and disorganised. The files appear to be PDFs of large spreadsheets in which adjacent pages have been separated so that it is impossible to read across the columns. There does not seem to be any coherent order to how entries are ordered, neither alphabetical or by magnitude of any particular value. This is an important issue as the Figures (eg Figure 2) do not detail any specific genes that are differentially expressed.

RESPONSE: We apologize for the formatting issue. The Supplementary Tables have now been resubmitted as Excel files via Figshare (<https://doi.org/10.6084/m9.figshare.29134847>) to ensure proper formatting.

Reviewer #2 (Remarks to the Author):

This manuscript presents a highly ambitious and methodologically sophisticated investigation that integrates spatial transcriptomics, single-nucleus RNA sequencing (snRNA-seq), lipidomics, and behavioral assays to explore regional and molecular host–parasite interactions during *Toxocara canis* neuroinvasion in a murine model. The study offers valuable insights into the immunopathological, endocrine, and neurological dimensions of neurotoxocariasis, addressing a critical gap in our understanding of parasitic infections of the central nervous system. The findings are novel and supported by a substantial body of data.

Nonetheless, I believe the manuscript requires major revision before it is suitable for publication. Specifically, the authors should temper some of the mechanistic interpretations, enhance the translational framing, and more clearly articulate the limitations of their experimental models and behavioral assays. My detailed concerns are outlined below:

RESPONSE: Thank you very much for your positive and constructive comments. We have made careful

revisions to address the issues and your concerns. Please find our responses to your specific criticisms in the following:

1. The manuscript draws strong conclusions linking *T. canis* larval presence to region-specific neuronal dysfunction and behavioral deficits. While spatial transcriptomic overlays and behavioral data suggest such associations, the evidence remains correlative. Establishing causality would require additional experimental controls—such as stereotactic injection of excretory-secretory (ES) products or immunological mimics—to disentangle the effects of larval activity from immune activation or systemic inflammation. Please clarify the limitations of inferring causality from co-localization and expand the discussion to include alternative interpretations of the observed phenotypes.

RESPONSE: Many thanks. We agree that our data are correlative. We have revised the text to temper causal language and have added a dedicated paragraph in the *Discussion* section on limitations. We explicitly state that while our multi-omics approach reveals strong spatial and molecular associations between larval presence, host response, and dysfunction, establishing direct causality (e.g., injecting naïve or recombinant ES proteins into the brains of mice) would require interventional studies (e.g., anthelmintic clearance or targeted immunomodulation) that are beyond the scope of this study.

2. The transcriptomic data implicating Tc-PEBP-1 and dauer-like gene programs are compelling. However, the conclusions regarding immune modulation and endocrine reprogramming rely heavily on *in vitro* findings and predicted molecular interactions. These should be explicitly framed as hypotheses requiring further *in vivo* validation. Incorporating functional assays—such as antibody blockade or RNAi-mediated knockdown of Tc-PEBP-1—would substantially strengthen the mechanistic claims.

RESPONSE: Thanks a lot for your critical comments. We have tempered our claims. As recommended by Reviewer #1, we have removed the *in vitro* macrophage stimulation assay. The retained data on the DAF-9–DAF-12 axis (now Fig. 5) is presented as evidence of a host-parasite endocrine dialogue that could facilitate persistence, framed explicitly as a hypothesis. We agree that *in vivo* validation is required in future work.

3. The rotarod and Morris Water Maze tests indicate mild-to-moderate impairments in motor learning and memory. However, small sample sizes and the absence of longitudinal tracking constrain the interpretability of these results. Additionally, the predator cue avoidance test yielded no significant differences, yet is discussed as corroborative. Please include power analyses for the behavioral outcomes, clarify the reliability and sensitivity of each assay, and moderate conclusions regarding neurocognitive deficits accordingly.

RESPONSE: We very much appreciate your comments and address them. A power analysis was performed to determine the minimum sample size required for the behavioural assays, which indicated mostly a minimum sample size of 2 mice per group. To account for potential variability and increase robustness, we used 12 mice per group (infected and uninfected) for all behavioural tests. With this increased power, the predator cue avoidance test conducted shows a significant reduction in avoidance behaviour (Fig. 6b, $p < 0.05$), corroborative results from other tests. We have clarified the interpretation, stating that the combined behavioural profile indicates deficits in motor learning, innate fear response and spatial memory, consistent with the region-specific transcriptional disruptions observed.

4. The manuscript situates the findings within a human health context, referencing disorders such as schizophrenia and Parkinson's disease. However, murine neurodevelopment, immune privilege, and glial architecture differ markedly from humans. The authors should more clearly position their findings as exploration within a model system and avoid direct extrapolation to human neuropathology without appropriate caveats regarding translational relevance and interspecies differences.

RESPONSE: We thank the reviewer for this important point and agree that careful distinction between model-based mechanistic insight and direct human extrapolation is essential. We have revised the manuscript to more explicitly frame our findings as arising from a murine experimental model, and to clarify that references to human neurological disorders (including schizophrenia and Parkinson's disease) are intended to provide biological context rather than direct clinical inference. Throughout the revised text, we emphasise that species-specific differences in neurodevelopment, immune privilege, and glial organisation limit direct translation from mouse to human.

Specifically, we have (i) strengthened statements in the *Introduction* and *Discussion* sections to indicate that this study is exploratory and hypothesis-generating, (ii) added explicit caveats regarding interspecific differences in neuroimmune architecture and disease manifestation, and (iii) revised wording to avoid any implication that the observed murine phenotypes directly model human neuropathology. Instead, we position our findings as defining neuroimmune and glial response pathways that may inform future investigations of parasite-associated neurological disease across species. These revisions ensure that the translational relevance of the work is presented in a measured and appropriately qualified manner, while retaining the broader One Health perspective that motivates the study.

5. The manuscript notes that mouse sex was not controlled for. This should be acknowledged as a limitation, with reference to known sex-specific differences in CNS immune responses and neuroinflammatory outcomes.

RESPONSE: This issue is acknowledged as a limitation in the *Discussion* section (lines 334-336): "the use of only female mice means potential sex-dependent differences in neuroimmune responses to *T. canis* are unexplored. Fourth, translational extrapolation from mice to humans is constrained by inherent interspecies differences in neurobiology and disease chronicity"

6. The spatial transcriptomic data would benefit from improved clarity. Specifically, a more explicit distinction between host and parasite signals would aid the reader's interpretation and enhance the impact of visualizations.

RESPONSE: Many thanks for your valuable suggestions. We have improved the visual distinction between host and parasite signals in several ways: 1) Fig. 1f: Added a heatmap specifically showing the *T. canis* molecular identity count per slice. 2) Fig. 2a-c: Provided high-magnification insets of larval niches with overlaid cell markers. 3) Supplementary Fig. 4: Clearly marked the positions of larvae. These changes make the spatial host-parasite interface clearer.

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

RESPONSE: Thank you very much for your co-review and for your valuable contributions to the peer-review process through this initiative. We have carefully considered all of the points raised in your report alongside the other reviewers' comments and have addressed them in the revised manuscript.

CONCLUDING REMARKS

In conclusion, we are grateful to your constructive and detailed feedback, which has substantially improved our manuscript. We have addressed each point raised and have added new data (uninfected snRNA-seq, brain lipidomics) as required, strengthening methodological rigour (expanded behavioural cohorts, clarified analyses), and enhancing our interpretations. We sincerely hope that the revised manuscript meets your approval.

18 March 2026

RE: Manuscript NCOMMS-25-39223A

Title: Spatial transcriptomic atlas of murine neurotoxocariasis reveals region-specific host responses and dysfunction in the brain

Thank you for the opportunity to revise our manuscript and for the additional constructive feedback. Below, we provide a point-by-point response to the remaining comments (authors' responses are provided below).

Reviewer #1 (Remarks to the Author):

The authors have made considerable revisions to their manuscript in response to the Reviewers' critiques, including conducting new experiments and re-drafting multiple sections of the text. Although much improved there are some remaining points requiring attention:

1. A central point was the absence of uninfected brain tissue controls from both spatial transcriptomics (Figure 2) and RNAseq (Figure 3). The authors responded by conducting RNAseq on uninfected brain tissue, but only show a very brief (and almost unintelligible) comparison of pathways added on to Figure 3 with one very general sentence in the Results (lines 166-168). What do the colors in Figure 3 j-l represent? In presenting Figure 2 the authors' text should clarify that only infected mice were analyzed.

RESPONSE: We thank the reviewer for highlighting the need for clearer presentation of infected versus control comparisons and for the constructive feedback.

We have revised the snRNA-seq Results section (lines 147–182) to explicitly present matched comparisons between *T. canis*-invaded and control samples (S4a/S4c, S5a/S5c, and S6a/S6c). These revisions include cell cluster identification, cellular state dynamics, cell-type composition, and differential gene expression at both global and cell-type-specific levels, providing a clear description of infection-associated changes. We now explicitly state in the Results, Methods, and figure legends that Fig. 2 includes only brain slices from *T. canis*-infected mice. In Fig. 3, panels j–l have been removed, cell-type proportions have been integrated into panels a–c, panels g–i have been revised to emphasise cell-type-specific transcriptional responses, and a new panel j has been introduced to integrate gene-level and pathway-level changes across infected and control conditions.

2. The text accompanying Figure 3 (lines 152-157) give detailed cellular analysis only on infected brain tissues and do not mention any comparisons with uninfected tissues. Here it is critical that the authors should give more prominence to the infected/uninfected comparison, extending Figure 3 j-l with specific gene levels as well as pathways, and adding similar analyses of imputed cell types, that would justify the statement on lines 170-171 about broader systemic effects of infection.

RESPONSE: We agree and have revised the manuscript to give greater prominence to infected versus matched control comparisons. The revised text (lines 149–180) explicitly presents these comparisons, including cell-type composition, cellular state transitions, and differential gene expression between *T. canis*-invaded samples and their corresponding controls (S4a/S4c, S5a/S5c, and S6a/S6c).

In addition, Fig. 3 has been revised to integrate gene-level and pathway-level information (panel j), together with cell-type-specific transcriptional responses (panels g–i), including inferred (imputed) cell populations. These revisions directly support our interpretation of broader, system-level effects of infection.

3. The authors need to double check their reference list. For example Ref 27 has no connection with studies on the fuzzy coat (lines 212-213), while in the Discussion (line 301) Ref 35 has no relation at all with the interaction of PEBP with macrophages.

RESPONSE: Thank you for your careful reading and for identifying these citation errors. We have thoroughly reviewed and corrected the citation or reference list. All references have been verified for accuracy and relevance.

(1) Ref. 27 has been replaced with Maizels (2013) to support statements on immune recognition and evasion. In addition, a recent spatial transcriptomics study (Han et al., 2025) has been incorporated to support the statement on limited leukocytic infiltration in *T. canis*-invaded rodent brains (line 112).

(2) Ref. 35 has been corrected to Zhou et al. (2022), which directly supports the interaction of *Tc*-PEBP with murine macrophages (line 305).

4. Line 268-270 (Figure 6): there seems a contradiction here; infected mice spend less time in the predator-scented compartment, hence their fear-related behavior is accentuated not suppressed. The Figure panels and legends are poorly explained, so perhaps this is just a presentational error, but given the prominence of the authors' claim that neurotoxocariasis has behavioral sequelae, it is essential that this is fully documented.

RESPONSE: Thank you for raising this important point. We confirm that this is not a presentational error. We agree that the observed reduction in time spent in the predator-scented compartment by infected mice suggests an accentuation, rather than suppression, of fear-related behaviour.

We have modified the Results and figure legend to clarify this interpretation and to improve the description of the behavioural data. We also acknowledge in the Discussion that the apparent divergence between olfactory-driven fear responses and learning/memory outcomes remains unresolved, likely reflecting region-specific effects (e.g. olfactory versus hippocampal circuitry), and requires further investigation.

5. Lines 460-462 : Why was it necessary to make a new NCBI database deposit for *Tc*-PEBP, (PV590067) when it already exists as U29761 from 1995, which is not acknowledged or the prior publication cited in the manuscript.\

RESPONSE: We thank the reviewer for this important point and apologise for not acknowledging the prior sequence (U29761), which has now been cited appropriately. The newly deposited sequence (PV590067) was generated from a geographically distinct isolate (Jiangxi, China) to support protein structure modelling. Although identical at the coding level to U29761, we deposited this sequence to ensure transparency and traceability of the material used in this study. This has now been clarified in the Data Availability statement and Supplementary Table 7, including that primers used to amplify PV590067 were designed based on the U29761 reference sequence.

Reviewer #2 (Remarks to the Author):

The authors have addressed my major concerns appropriately; therefore, I recommend the manuscript for acceptance in this respected journal.

RESPONSE: Thank you very much for your time, expertise, and thoughtful feedback throughout the review process. We truly appreciate your constructive comments, which have helped us strengthen the manuscript.

Reviewer #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

RESPONSE: Thank you again for your thoughtful co-review and for your valuable contributions to the peer-review process. We truly appreciate the time and effort you have dedicated to helping us improve our manuscript.

CONCLUDING REMARKS

In conclusion, we are grateful for your positive and insightful feedback, which has been valuable in helping us further enhance our manuscript. We have thoroughly addressed all points raised and revised the manuscript, figures, and figure legends to improve clarity and interpretation. We hope that the revised manuscript now meets your approval.
