Course of the Short Note sn20250916-4r

Short Note submitted on: Monday February 17, 2025

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Editorial response: Monday February 17, 2025

> Subject: decision on your Short Note submission
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> Dear Authors,
>
> Thank you for submitting your manuscript "Experimental setup to investigate the emotional valence of olfactory stimuli in sheep (Ovis Aries)". We appreciate your commitment to advancing open science by sharing your results.
>
> Your manuscript shows significant promise after review by our expert panel, but requires revisions to be accepted. Therefore, we invite you to resubmit your manuscript after addressing the reviewers' comments and suggestions.
>
> Below yopu will find detailed feedback from each reviewer. We encourage you to carefully consider this feedback and make appropriate revisions to your manuscript.
>
> For your resubmission, please use the dedicated "authors'response space" in the submission system to systematically address each reviewer's comment. In this section, clearly detail how you have incorporated their feedback or, if you disagree with any points, provide a well-reasoned justification for not making specific changes. This approach ensures a transparent and direct link between each criticism and your response or rebuttal.
>
> You have until April 15 to submit your revised manuscript. We hope you will find the reviewers' feedback constructive and look forward to your resubmission. Please do not hesitate to contact us if you have any questions or require further clarification on the reviewers' comments.
>
> Thank you for considering Short Notes as a platform to share your work. We wish you a successful revision process and look forward to receiving your improved manuscript.
>
> Sincerely,
>
> The Short Notes editorial board

Reviewer 1 - Review :

Quality of the writing: no
The authors do not answer to their question. There are no experimental data to validate their experimental set-up, just "expected data" or "expected results". No explanations were provided concerning how they obtained these "expected data" on Figure 2C and D. The only data showed in the paper concerned the habituation phase but no statistical analyses were provided.

Quality of figures and additional documents: no
For Figure 1C, the dimensions of the experimental environment (including the birtual zone mentioned as "close to food ressource" or "close to social ressource) are missing which is essential for reproducibility.
There are no statistical analyses on Figure 2A and B.
Figure 2 C and D are "expected" results coming from apparently nowhere (no explanations provided on the origin of these "expected data").

Quality of the experimental design: no
The aim of this study, as indicated in the title, is to present an experimental set-up that allows investigating the emotional valence of olfactory stimuli in sheep. The habituation phase is well described but that is the only real data presented in this short-note. No odours where tested in this experimental set-up. They proposed "expected data". The authors thus cannot answer to their hypotheses and cannot validate their experimental set-up as a real way to investigate the emotional valence of odours.
Furthermore, they expected the ewes to increase their intake in the presence of an odour with a positive valence, as I said they did not test it and if they had wanted to, they cannot as the ewes were not ad libitum during the test (they ate the 300g of pellets provided). So, there is a problem in the experimental design here.
Another major problem is that there are no statistical analyses in this short-note.

Quality of the reporting: no
Some informations are missing as the temperature, the lightening, the time of testing, the familiarity or not of the animals used as "social ressource", the cleaning / ventilation of the PMMA box between two tests to avoid social odours or accumulation of the odour that can be tested withthis set-up (they did not but it might be a problem with such a set-up).
The description of the beahviours in Table 1 is not precise enough : "moves in the corridor" means that they are walking/runing but not just moving their head, the zone "close to the food" and "close to the social ressource" are not defined, and I am wondering how the experimenter can see wether there were pellets in the mouth if the entire head is in the trough so it might be rephrased.
There are no statistics for the real data and no explanations provided to know how the "expected data" were obtained.

Final reviewer decision: no
This short-note cannot validate the experimental set-up proposed as a way to investigate the emotional valence of olfactory stimuli as the authors did not test any odour in the study. They were expected results which is not enough to be published.

The data presented in the short-note refers to the habituation to the experimental set-up, but no statistical analyses were provided.
These data showed that ewes ate all the pellets ofered during the test which is a porblem as the authors supposed that an odour with a positive valence will increase the amount of feed eaten during the test.
The "expected results" were not explained with a predictive model or with references to the existing literature. They cannot be considered as serious for publication.

Reviewer 2 - Review :

Quality of the writing: yes

Quality of figures and additional documents: no
fig 2c and 2d: What do these plots represent? How are the quantity of pellets ingested and the number of vocalisations estimated?

no statistical analysis integrated

Quality of the experimental design: no
no statistical analysis mentioned

Quality of the reporting: no
data treatments are not detailed.
Authors "hypothesise that this experimental setup will reduce individual variability, improve reproducibility of data" but according to graph 2, there is a high degree of inter-individual variability.
The experimental setup responds to the refinement of the 3Rs, but for the reduction it is less obvious.

Final reviewer decision: to be discussed
Interesting and well-written article, but it lacks the statistical processing applied.

request for further details: 1) what are the characteristics of ewes (page 6)that don't approach the box? are they more emotional?
2) the authors write "For testing social buffering with our experimental setup, we simply reduce the level of social resource by reducing the number of congeners in the presence of a negative valence OS". but it may not just be a question of numbers. some animals have more skills than others, and affinities between conspecifics may also be important.

Reviewer 3 - Review :

Quality of the writing: no

Please, see below for details.

Quality of figures and additional documents: no
- Some captions lack details that would improve understanding, particularly for Table 1.

- Figure 2A could be made easier to analyze by providing separate diagrams for lambs and ewes to better adapt the scale (as lambs consume 200g, compared to 300g for ewes). Additionally, there is an unnecessary "and" at the end of the Figure 2A caption.

- In Figure 2, statistical information (e.g., significance levels or p-values) could be integrated within the boxplots and captions.

Quality of the experimental design: no
The study aims to validate an experimental approach for investigating the emotional component of various olfactory stimuli by describing an experiment where this method has been successfully applied. However, to properly establish proof of concept, the Materials and Methods section, as well as the Results, need further details.

It appears that the authors have mixed the results of an experimental study with their objective of validating an experimental approach to assess the emotional valence of olfactory stimuli. This leads to some confusion, as both the experimental results and the methodological validation seem insufficiently detailed.

- Descriptive statistics of the population are missing (e.g., median age, minimum and maximum age for ewes, and for 3- and 4-month-old lambs).
- The statistical tests used to compare the training and experimental periods (and potentially different groups) are not mentioned. It may be helpful to include boxplots from the training period within Figures 2A and 2B.
- The rationale behind the expected results regarding the quantity of pellets ingested and the number of vocalizations (Figures 2C and 2D) should be explained.

Quality of the reporting: no
The description of the experimental approach is sometimes difficult to follow, and some explanations are missing:

- The olfactory stimuli (OS) used in the study are not clearly specified. The text suggests that different boxes were used for each OS, but this is not explicitly detailed.
- Certain justifications are lacking. For instance, why did the authors choose 11 cm as the distance between the plastic trough opening and the OS?
- The authors provide three reasons for placing the OS behind the food but only list two.
- Since the PMMA boxes were cleaned before being used for a new OS, the cleaning product used should be specified, as it could interfere with the olfactory stimuli depending on its composition.
- In the Experimental Setup section, the sentence describing the number of companions for lambs and ewes is unclear.

- The duration of the training period is described as "5 to 10 days," but the exact number of days or weeks should be specified.
- The experiment appears to have lasted four weeks, but the rationale behind selecting time points J18, J22, and J25 should be clarified.
- The definition of "volunteer" in the context of companion animals accompanying the tested subjects needs to be specified.

Final reviewer decision: to be discussed
This study adheres to the 3R principles by proposing an experimental approach that reduces animal stress, improves welfare, and minimizes confounding factors when studying behavioral responses to olfactory stimuli.

To support its objectives, the study presents the validation of an experimental method through the description of a successful application. However, to fully demonstrate proof of concept, the Materials and Methods and Results sections require additional details, including statistical analyses, figure annotations, and a discussion of limitations.

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Author's first response: Friday March 28, 2025

Reviewers

Inès ACHIN
Nouzilly, 37380, France
Tel: +33 2 47 42 78 53
Ines.achin@inrae.fr

Subject: Response to Reviewer Comments
Dear Reviewers,
Thank you for your insightful comments regarding our study. We appreciate your feedback and the opportunity to clarify our article.
The primary objective of this Short Note is to introduce our methodological approach for assessing the impact of olfactory stimuli on the behavioural response of sheep within a non stressful context. As such, the article focuses on describing the experimental setup rather than presenting specific results or statistical analyses related to particular odours. We acknowledge that the absence of detailed results and statistical analyses may be perceived as a limitation. However, to demonstrate the relevance of this experimental setup, we included all the behavioural responses measured in control situation. The impact of odours with contrasting emotional valences was explored with this methodological framework. These results are presented in an open-access article (https://doi.org/10.5281/zenodo.14650739, https://doi.org/10.57745/XSND95).

Given that our methodological details could not be extensively described in our previously submitted research articles, we opted for a Short Note format to specifically highlight our experimental approach. We believe that this contribution enhances the 3Rs framework by providing an alternative method for future studies in this field.
We sincerely appreciate your constructive feedback and remain available for any further clarifications.
Best regards,
ACHIN Inès

Reviewer 1 - Review :

Quality of the writing: no
The authors do not answer to their question. There are no experimental data to validate their experimental set-up, just "expected data" or "expected results". No explanations were provided concerning how they obtained these "expected data" on Figure 2C and D. The only data showed in the paper concerned the habituation phase but no statistical analyses were provided.
We appreciate the reviewer's constructive feedback and have addressed the concerns raised. Regarding the quality of the writing, we have carefully revised the manuscript to improve clarity and precision throughout. In response to the comment about not answering the research question, we agree with this observation. As a result, we have updated the title and the aim of the paper to clearly reflect its focus on presenting the methodological approach, rather than on analyzing specific results.
The concern about the lack of experimental data validating the setup is valid. We have removed the illustrations of expected data (Figures 2C and 2D) and excluded them from the revised version of the manuscript. Additionally, we have revised the manuscript to emphasize the methodological aspects only.

Lastly, concerning the observation that the only data presented in the manuscript relate to the habituation phase without statistical analysis, we clarify that the experimental data obtained with this setup have been submitted for publication and are available on Zenodo at the following link: https://doi.org/10.5281/zenodo.14650739.
Quality of figures and additional documents: no
For Figure 1C, the dimensions of the experimental environment (including the birtual zone mentioned as "close to food ressource" or "close to social ressource) are missing which is essential for reproducibility.
In response to the comment regarding the quality of figures and additional documents, we acknowledge the importance of providing complete information for reproducibility. Specifically, for Figure 1C, we have now included the dimensions of the experimental environment directly within the figure.
There are no statistical analyses on Figure 2A and B.
We agree with the reviewer's comment regarding the absence of statistical analyses (Figure 2). As a result, this figure has been removed from the manuscript. The primary objective of this article is to present the methodological approach. The application of the experimental data is presented in a separate research article, where the impact of odors with contrasting emotional valences is analyzed. This open-access article provides further details on the data analysis. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739 .
Figure 2 C and D are "expected" results coming from apparently nowhere (no explanations provided on the origin of these "expected data").

This figure has been removed from the manuscript.
Quality of the experimental design: no
The aim of this study, as indicated in the title, is to present an experimental set-up that allows investigating the emotional valence of olfactory stimuli in sheep. The habituation phase is well described but that is the only real data presented in this short-note.
We agree with the reviewer's comment regarding the focus on the habituation phase as the only data presented in this short note. The primary objective of this study is indeed to present an experimental setup that minimizes potential experimental biases, which could generate confounding emotional responses that might interfere with the emotional responses related to the odors themselves. By ensuring a well-controlled habituation phase, we aim to eliminate these biases and provide a clear foundation for future studies investigating the emotional valence of olfactory stimuli in sheep.
No odours where tested in this experimental set-up. They proposed "expected data". The authors thus cannot answer to their hypotheses and cannot validate their experimental set-up as a real way to investigate the emotional valence of odours.
We agree with the reviewer's comment that no odors were tested in this experimental setup, as the current manuscript focuses on presenting the methodology rather than experimental results. The original results, including those involving odors with varying emotional valences, are part of separate publication. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739 .
Furthermore, they expected the ewes to increase their intake in the presence of an odour with a positive valence, as I said they did not test it and if they had wanted to, they cannot as the ewes were not ad libitum during the test (they ate the 300g of pellets provided). So, there is a problem in the experimental design here. Another major problem is that there are no statistical analyses in this short-note.
We agree with the reviewer's comment that the ewes could not increase their intake due to the fixed amount of 300g of pellets provided during the test. As you pointed out, this does limit the ability to assess food intake as an indicator of emotional valence. To address this, we are focusing on alternative behavioral criteria, such as the duration of time spent with the head in the feeder or proximity to the odor. One possible solution to address this limitation in future studies would be to either provide an additional food resource or conduct a shorter test duration, allowing for a more reliable measure of the effect of the odor on the ewes' behavior. Additionally, we acknowledge the absence of statistical analyses in this short note. As previously mentioned, the goal of this manuscript is to present the experimental setup
Quality of the reporting: no
Some informations are missing as the temperature, the lightening, the time of testing, the familiarity or not of the animals used as "social ressource", the cleaning / ventilation of the PMMA box between two tests to avoid social odours or accumulation of the odour that can be tested with this set-up (they did not but it might be a problem with such a set-up).
Done line 84. page 3
Done line 104. Page4.
The description of the beahviours in Table 1 is not precise enough : "moves in the corridor" means that they are walking/runing but not just moving their head, the zone "close to the food" and "close to the social ressource" are not defined, and I am wondering how the experimenter can see wether there were pellets in the mouth if the entire head is in the trough so it might be rephrased.
Thank you for your comment. You are right that the term "moves in the corridor" could be misleading, as the space is too narrow for running. We will clarify that the frequency of zones crossed is used as an indicator of the animal's agitation. We have incorporated this clarification into a new section,

Measurement of Behaviour, on page 7.
Regarding the observation of mouth movements, we will specify that this is conducted using the front camera, which provides a clear view of the animal's mouth, allowing the experimenter to accurately assess these movements (see Figure 1E for the front camera view).

There are no statistics for the real data and no explanations provided to know how the "expected data" were obtained.
We agree with the reviewer's comment that no odors were tested in this experimental setup, as the current manuscript focuses on presenting the methodology rather than experimental results. The original results, including those involving odors with varying emotional valences, are part of separate publication. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739 .
Final reviewer decision: no
This short-note cannot validate the experimental set-up proposed as a way to investigate the emotional valence of olfactory stimuli as the authors did not test any odour in the study. They were expected results which is not enough to be published.
We agree with the reviewer's comment that no odors were tested in this experimental setup, as the current manuscript focuses on presenting the methodology rather than experimental results. The original results, including those involving odors with varying emotional valences, are part of separate publication. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739.
Additionally, we have modified the title of the manuscript to better reflect the focus on behavioral responses, rather than on testing odors themselves. We believe that this clarifies the intent of the short-note, which is to provide a methodological approach for investigating the behavioral response to olfactory stimuli in sheep.
The data presented in the short-note refers to the habituation to the experimental set-up, but no statistical analyses were provided.
We agree with the reviewer's comment regarding the absence of statistical analyses for the habituation phase. The data presented in the short-note primarily focuses on the methodological approach, with the original results, including those of the habituation phase, being published separately. For more detailed information on the results for the ewes, including statistical analyses, please refer to the following Zenodo link: https://doi.org/10.5281/zenodo.14650739 .
Additionally, we are in the process of publishing results concerning the habituation phase for lambs in a separate document, which will be accessible once published.
These data showed that ewes ate all the pellets ofered during the test which is a porblem as the authors supposed that an odour with a positive valence will increase the amount of feed eaten during the test.
We agree with the reviewer's comment regarding the animals eating all the pellets offered during the test. As noted, the ewes consumed the full amount of feed provided, which makes it impossible to observe an increase in food intake in the presence of a positive-valence odor. However, as the animals consumed all of their ration, we are focusing on alternative behavioral indicators. Specifically, we expect that the presence of a positive-valence odor would lead to an increase in the time spent with the head in the trough or box.
The "expected results" were not explained with a predictive model or with references to the existing literature. They cannot be considered as serious for publication.
We agree with the reviewer's comment regarding the absence of statistical analyses (Figure 2). As a result, this figure has been removed from the manuscript.

Reviewer 2 -:
Quality of the writing: yes
Thank you for your positive feedback on the quality of the writing. We appreciate your thoughtful review and are glad that the manuscript met the expected writing standards. Your comments are very helpful in improving the clarity and quality of our work. Thank you again for your time and input.
Quality of figures and additional documents: no
fig 2c and 2d: What do these plots represent? How are the quantity of pellets ingested and the number of vocalisations estimated?
Thank you for your comment. Figures 2C and 2D initially represented the expected results, specifically the estimated quantity of pellets ingested and the number of vocalizations. However, in response to reviewers feedback, we have removed these graphs from the manuscript as they do not align with the primary aim of presenting the experimental setup.
no statistical analysis integrated
We agree with the reviewer's comment regarding the absence of statistical analyses (Figure 2). As a result, this figure has been removed from the manuscript. The primary objective of this article is to present the methodological approach. The application of the experimental data is presented in a separate research article, where the impact of odors with contrasting emotional valences is analyzed. This open-access article provides further details on the data analysis. For more information, please refer to the following links: https://doi.org/10.5281/zenodo.14650739 .
Quality of the experimental design: no
no statistical analysis mentioned
Quality of the reporting: no
data treatments are not detailed.
We agree with the reviewer's comment regarding the absence of statistical analyses (Figure 2). As a result, this figure has been removed from the manuscript. The primary objective of this article is to present the methodological approach. The application of the experimental data is presented in a separate research article, where the impact of odors with contrasting emotional valences is analyzed. This open-access article provides further details on the data analysis. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739 .
Authors "hypothesise that this experimental setup will reduce individual variability, improve reproducibility of data" but according to graph 2, there is a high degree of inter-individual variability.
The experimental setup responds to the refinement of the 3Rs, but for the reduction it is less obvious.
Thank you for your comment. We acknowledge that Figures 2A and 2B show a high degree of inter-individual variability. However, our experimental setup is designed to create a conflict of motivation between social and food resources. This approach allows us to identify different populations based on their motivation, whether social or food-related, which explains this variability. Rather than being a limitation, this variability is an important feature that helps differentiate animals with different motivational profiles. This aspect is detailed in the publication on ewes, which is available in open access on Zenodo.
Final reviewer decision: to be discussed
Interesting and well-written article, but it lacks the statistical processing applied. request for further details: 1) what are the characteristics of ewes (page 6) that don't approach the box? are they more emotional?
We have added ACP (figure 2) The results suggest that ewes and 4-month-old lambs who do not

approach the box tend to cluster separately, indicating behavioral differences from the rest of the group. These individuals do not vocalize and are often observed in the entrance zone. Instead, they remain close to their conspecifics, which suggests a stronger social motivation rather than a primary drive for food. This reinforces the idea of individual variability in motivation, ranging from food-oriented to socially-oriented profiles.
2) the authors write "For testing social buffering with our experimental setup, we simply reduce the level of social resource by reducing the number of congeners in the presence of a negative valence OS". but it may not just be a question of numbers. some animals have more skills than others, and affinities between conspecifics may also be important.
We agree that social buffering is influenced not only by the number of conspecifics but also by individual social skills and affinities between animals. However, assessing these affinities would require a preliminary characterization of the social network within the group before testing. Several studies have investigated social buffering by using familiar conspecifics and varying their number, which aligns with our approach
Done line 231. Page 9.
Reviewer 3

Quality of the writing: no
Please, see below for details.

Quality of figures and additional documents: no
- Some captions lack details that would improve understanding, particularly for Table 1.
Table 1 has been removed, and the behaviors have been detailed in a Measurement of Behaviors section on page 7.
Figure 2A could be made easier to analyze by providing separate diagrams for lambs and ewes to better adapt the scale (as lambs consume 200g, compared to 300g for ewes). Additionally, there is an unnecessary "and" at the end of the Figure 2A caption.
Figure 2 has been removed, but we appreciate the reviewer’s comment. Thank you for your suggestion.
- In Figure 2, statistical information (e.g., significance levels or p-values) could be integrated within the boxplots and captions.
We agree with the reviewer's comment regarding the absence of statistical analyses (Figure 2). As a result, this figure has been removed from the manuscript. The primary objective of this article is to present the methodological approach. The application of the experimental data is presented in a separate research article, where the impact of odors with contrasting emotional valences is analyzed. This open-access article provides further details on the data analysis. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739 .
Quality of the experimental design: no
The study aims to validate an experimental approach for investigating the emotional component of various olfactory stimuli by describing an experiment where this method has been successfully applied. However, to properly establish proof of concept, the Materials and Methods section, as well as the Results, need further details.
It appears that the authors have mixed the results of an experimental study with their objective of validating an experimental approach to assess the emotional valence of olfactory stimuli. This leads to some confusion, as both the experimental results and the methodological validation seem insufficiently

detailed.
Reviewer 3 has correctly understood our approach. We acknowledge that we attempted to improve the clarity of the short note.
We agree with the reviewer's comment regarding the absence of statistical analyses (Figure 2). As a result, this figure has been removed from the manuscript. The primary objective of this article is to present the methodological approach. The application of the experimental data is presented in a separate research article, where the impact of odors with contrasting emotional valences is analyzed. This open-access article provides further details on the data analysis. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739.
This experimental setup has been used with both ewes and lambs to test different odors, including water (control), cadaverine, wolf feces, and orange scent. These exposure sessions were conducted over 5 weeks for ewes and 4 weeks for lambs (figure 2). Regardless of the specific experiment, all animals were systematically exposed to a control condition (water). The validation of our experimental design relies on these results, demonstrating the consistency of behavioral responses by comparing control conditions across different experiments This is explained in figure 2

- Descriptive statistics of the population are missing (e.g., median age, minimum and maximum age for ewes, and for 3- and 4-month-old lambs).
- The statistical tests used to compare the training and experimental periods (and potentially different groups) are not mentioned. It may be helpful to include boxplots from the training period within Figures 2A and 2B.
- The rationale behind the expected results regarding the quantity of pellets ingested and the number of vocalizations (Figures 2C and 2D) should be explained.
We agree with the reviewer's comment regarding the absence of statistical analyses in Figures 2C and 2D. As a result, this figure has been removed from the manuscript. The primary objective of this article is to present the methodological approach, and we aim to provide a clearer representation of the experimental setup and the expected behavioral outcomes. The application of the experimental data, including statistical analysis, is presented in a separate research article, where we analyze the impact of odors with contrasting emotional valences. This open-access article provides further details on the data analysis. For more information, please refer to the following link: https://doi.org/10.5281/zenodo.14650739.
Quality of the reporting: no
The description of the experimental approach is sometimes difficult to follow, and some explanations are missing:
- The olfactory stimuli (OS) used in the study are not clearly specified. The text suggests that different boxes were used for each OS, but this is not explicitly detailed.
Thank you for your comment. We apologize for the lack of clarity regarding the olfactory stimuli used. The following odors were used during the experiments:
Water (control)
Orange
Cadaverine
Feces from a wolf (3g)
Feces from a wolf (15g)
The results can be found in the open-access publication available on Zenodo:

https://doi.org/10.5281/zenodo.14650739 .
- Certain justifications are lacking. For instance, why did the authors choose 11 cm as the distance between the plastic trough opening and the OS?
Thank you for your comment. The choice of 11 cm as the distance between the opening and the trough was made based on the size of the animals' heads. We wanted to ensure that the animal's head would be completely inside the box to allow for optimal exposure to the odor
- The authors provide three reasons for placing the OS behind the food but only list two.
We apologize for the confusion. You are correct that we originally provided two reasons for placing the olfactory stimulus (OS) behind the food. The revision has been made on line 97, page 4.
- Since the PMMA boxes were cleaned before being used for a new OS, the cleaning product used should be specified, as it could interfere with the olfactory stimuli depending on its composition.
Thank you for your comment. We agree that specifying the cleaning process is important. The PMMA boxes were cleaned with water before being used for a new olfactory stimulus. We have updated the manuscript accordingly, with modifications made to line 104. Page 4.
- In the Experimental Setup section, the sentence describing the number of companions for lambs and ewes is unclear.
Thank you for your comment. The updated sentence is now found in line 123 on page 6.
- The duration of the training period is described as "5 to 10 days," but the exact number of days or weeks should be specified.
Thank you for your comment. The updated information can now be found in line 137 page 6.
The experiment appears to have lasted four weeks, but the rationale behind selecting time points J18, J22, and J25 should be clarified.
Thank you for your comment. Figure 2 has been removed from the manuscript. The detailed analysis of the experimental data is available in our open-access publication on Zenodo: https://doi.org/10.5281/zenodo.14650739.
The definition of "volunteer" in the context of companion animals accompanying the tested subjects needs to be specified.
Thank you for your comment. In this context, "volunteer" refers to animals that present themselves spontaneously for the test. At this step, we suggest that the order of passage be determined by respecting the order in which animals voluntarily present themselves. Done line 140 p.6
Final reviewer decision: to be discussed
This study adheres to the 3R principles by proposing an experimental approach that reduces animal stress, improves welfare, and minimizes confounding factors when studying behavioral responses to olfactory stimuli.
To support its objectives, the study presents the validation of an experimental method through the description of a successful application. However, to fully demonstrate proof of concept, the Materials and Methods and Results sections require additional details, including statistical analyses, figure annotations, and a discussion of limitation

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Revised version of the Short Note submitted on: Friday March 28, 2025

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2nd editorial response: Sunday June 15, 2025

Subject: Conditional Acceptance – Final Revisions Required for Your Short Note

Dear Authors,

Please find below the final editorial decision and the second review round from the reviewers.

=>Your Short Note has been accepted with minor revisions how can the authors can attribute vocalisation with certainty to one individual or another?
Justify why the habituation phase differ for ewes and lambs (no habituation to the experimenter, lower number of days in the setup). Add a table with each step of the habituation process, with the corresponding day, for ewes vs. for lambs.
Habituation lasted 5 days for ewes but you consider 4 exposures in the PCA. Justify. You consider 3 exposures for lambs in the PCA while the duration of the habituation detailed in the "strategy..." section is not clearly mentioned.
l181 : orientation of the muzzle or localisation of the muzzle? unclear
On Fig2A, the time diagram seems to show that there were 2 habituation phase for the ewes. Explain and add this in the M&M.

Final reviewer 2 decision: to be discussed
Adjustments have been made, but the objectives of this work still need to be specified in the title and introduction. This work, which focuses on habituation, must be clearly distinguished from the work already published in the form of a preprint (concerning the emotional evaluation of the OS). I would appreciate an explanation of the publication strategy for this work as, in the responses to the reviewers, it is mentioned that an article is being prepared on the “habituation” part and that the results will be detailed there ("Additionally, we are in the process of publishing results concerning the habituation phase for lambs in a separate document, which will be accessible once published.").
In terms of materials and methods, the short note needs to focus on the habituation phase and provide all the necessary details (why 2 habituation phases for the ewes? what is done and on what day? which days are included in the PCA and why? etc.).
For the results, there is a lack of data that can be extracted from the PCA to enable the reader to interpret the relevance of the results. The PCA is not sufficient to conclude that behaviours are stable over days, during the habituation. Instead, it can be used to produce synthetic variables on which further analysis should be carried out.

Reviewer 3 - Review :

Quality of the writing: yes

Quality of figures and additional documents: yes

Quality of the experimental design: yes

Quality of the reporting: yes

Final reviewer 3 decision: yes
thanks to the authors for this revised version

Author's 2nd response: Wednesday July 16, 2025

Reviewer 1 - Review :

Quality of the writing: yes

Quality of figures and additional documents: no
The figure 2 needs further explanations within the text: the authors mention "Dimension 1" and "Dimension 2" but these terms are not clearly explained in the text nor within the caption of the figure. Cited Line 193-198 and add in legend line 212-217.

Quality of the experimental design: yes
The authors significantly improved the M&M and the result sections, in particular by reformulating the aim of the study. The previous concern (mix of the results of an experimental study with their objective of validating an experimental approach to assess the emotional valence of olfactory stimuli) has been corrected as the study has became a proposed experimental design to to explore the emotional valence of an olfactory stimulus.

Quality of the reporting: yes

Final reviewer 1 decision: to be discussed
The short note has been considerably improved by only describe the experimental set up to assess emotional valence of olfactory stimuli in sheeps. The previous results of an example of a study using this

design was insufficiently described, so the authors chose to only described the validation of the design.

My final decision is "to be discussed" but could be "accepted with minor revisions" :
- The figure 2 needs further explanations within the text: the authors mention "Dimension 1" and "Dimension 2" but these terms are not clearly explained in the text nor within the caption of the figure. DONE
- Maybe the title would be modified as "Proposed/description of an experimental setup to investigate the impact of olfactory stimuli on behavioural responses in sheep (Ovis aries)" DONE

Reviewer 2 - Review :

Quality of the writing: no
Some improvements have been made in this revised short note and I would like to thank the authors. However, the title and aim of this paper remain unclear. Please mention in the title and objectives that this short note aims to present an experimental design that minimizes potential experimental bias by focusing on the habituation phase. DONE Line 66
Provide all the data and results necessary to prove your assumptions = reduce individual variability - improve reproducibility. Please justify why you are in the process of publishing the results of the habituation phase in a separate document. These results should be included in this short-note to demonstrate that the setup enhances the 3Rs. Result published in Animal journal
Please remove the fact that the setup will measure emotion-related behaviours induced by the OS as this is not what the authors are addressing in this short note. However, you can clearly mention in the text that the results concerning the emotional valence of different OS are available as a preprint online. DONE

Quality of figures and additional documents: no
The time diagrams (in fig 2A and 2B) are too small to be understandable. They should be enlarged and included in the materials and methods. DONE

Quality of the experimental design: no
Please add the number of animals per experiment, per sex. DONE table 1
The data treatment need to be detailed: mention clearly in the text the days included in the analysis and justify; Add more details about the CPA (software, Pearson/Spearman/Covariance).
l191 - after the training period or at the end of it? mention the day. DONE line 189
If the question is about the consistency of the behavioural responses during the habituation phase, I am wondering why the authors did not test for a day effect with a model that also includes the experiment and the sex. Please justify. They can use the PCA to extract synthetic variables from the 29 measures and then, perform the analysis on these synthetic variables. They cannot conlude about a day effect only with the PCA. Thank you for the suggestion. However, from our point of view, the synthetic variable does not capture the full variability of the data, and the PCA does not allow us to cluster the evidence related to the day factor.

Concerning the PCA, provide the eignevalue and eigenvectors so that the reader could interpret the

dimensions showed on the graph by itself. Cited Line 193-198 and add in legend line 212-217.

Quality of the reporting: no
As the authors clarified the aim of the short-note and mentioned that it is a focus on the habituation phase. Please, remove all the text concerning the different OS tested as it is not the aim of this short-not (refer briefly to it when needed, and mention the preprint online for more details). DONE
Add more details about the habituation phase and the control condition:quantity of water put on the coton pad? tap water? detailed method for each step of the habituation phase (duration of familiarization with the experimenter, experimenter familiar or not to the sheep, experimenter moving or not/speaking or not, how the reduction of the distance was done, movement from one room to another with 3 to 5 animals ... the same groups in every step? the same group between the different days of habituation?). DONE L108
Thus, describe clearly and precisely each day of habituation, with the measures performed and the criteria to validate each step.Define what is "agitation" in the test corridor regarding the measures?
Specify in the caption to photo 1D that this illustrates habituation day 3, in group, but that the sheep were then tested alone the test corridor for days 4-5. The criteria for validation are: eating from the bucket and putting the head into the box (LINES 159–161).
Was there any social competition to access the PMMA box at day 3 of habituation? Did the authors note whether all the sheep of the group visited the PMMA box on that day? Yes is a criteria for validation.
Please rephrase the sentence l 152-154 and clarify l 154-155 (how was done the training? day of habituation concerned by these events?). Please add the information concerning the amount of water put on the coton pad l108. DONE
During the habituation phase, the "social ressources" are not in the "social ressource zone" but sheep are tested in group. Clarify the "material and method" during the habituation phase and the one for the following experimental phase (not included in this short-note but in the preprint online). I don’t understand.
Provide the lightening and temperature conditions for each experiment, during the habituation phase. L84-87
Provide the size/dimensions of the 3 virtual zones. DONE Line 116
Provide the timing of the experiment during the day (animals tested in the morning, afternoon, or all day long). DONE Line 137
Add the number of males and females in each experiment, in table 1. DONE
Justify the dimension of the PMMA box in regard to the size of the head of a sheep (adult one ?), and maybe also the size of the trough. Thank you for your comment. The choice of 11 cm as the distance between the opening and the trough was made based on the size of the animals' heads. We wanted to ensure that the animal's head would be completely inside the box to allow for optimal exposure to the odor
Justify why putting the OS in the PMMA box, with the food, as the authors mentioned that they wanted to measure behaviours due to the OS only.It could have been preferable to separate them completely. The OS (olfactory stimuli) are placed behind the food to motivate the animal to put its head into the box. If the OS were placed near the conspecific, there would be a risk of emotional contagion.
The PMMA box was cleaned with a dry cloth between 2 animals. The authors mentioned in the text that a new PMMA box was used for each OS but also mentioned in the response to reviewer 3 that the PMMA box was cleaned with water between different OS. It is quite confusing but can be removed as the short-note focuses on the habituation phase. DONE

Clarify where the camera was placed regarding the trough, the animal The camera is placed behind the PMMA box
Clarify whether "Time spent with the head inside the box" includes "time spent with the head inside the box without eating" as soon as it is mentioned.
Rephrase "time spent during the 1rst feeding" as well as "time spent during the 1st occurence in the social/food zone". DONE
Vocalizations are measured in a social context => how can the authors can attribute vocalisation with certainty to one individual or another? With camera
Justify why the habituation phase differ for ewes and lambs (no habituation to the experimenter, lower number of days in the setup). Add a table with each step of the habituation process, with the corresponding day, for ewes vs. for lambs. No, because we are limited by the FC3R format.
Habituation lasted 5 days for ewes but you consider 4 exposures in the PCA. Justify. You consider 3 exposures for lambs in the PCA while the duration of the habituation detailed in the "strategy..." section is not clearly mentioned. Sorry for the confusion, but it is clearly stated that the days used for the PCA are not those of the habituation phase (Fig. 2).
l181 : orientation of the muzzle or localisation of the muzzle? The unclear location of the animal between the two zones is determined based on the orientation of its muzzle—either toward the social zone or the food zone."
On Fig2A, the time diagram seems to show that there were 2 habituation phase for the ewes. Explain and add this in the M&M. To ensure that the first presentation of the odor had no effect (detailed in the article on the animal).
Final reviewer 2 decision: to be discussed
Adjustments have been made, but the objectives of this work still need to be specified in the title and introduction. This work, which focuses on habituation, must be clearly distinguished from the work already published in the form of a preprint (concerning the emotional evaluation of the OS). I would appreciate an explanation of the publication strategy for this work as, in the responses to the reviewers, it is mentioned that an article is being prepared on the “habituation” part and that the results will be detailed there ("Additionally, we are in the process of publishing results concerning the habituation phase for lambs in a separate document, which will be accessible once published."). The short note focuses not only on habituation, but also on the PMMA box.
In terms of materials and methods, the short note needs to focus on the habituation phase and provide all the necessary details (why 2 habituation phases for the ewes? what is done and on what day? which days are included in the PCA and why? etc.). Done in figure 2
For the results, there is a lack of data that can be extracted from the PCA to enable the reader to interpret the relevance of the results. The PCA is not sufficient to conclude that behaviours are stable over days, during the habituation. Instead, it can be used to produce synthetic variables on which further analysis should be carried out.

Reviewer 3 - Review :

Quality of the writing: yes

Quality of figures and additional documents: yes

Quality of the experimental design: yes

Quality of the reporting: yes

Final reviewer 3 decision: yes
thanks to the authors for this revised version

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Revised version of the Short Note submitted on: Friday September 12, 2025

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Final editorial response: Tuesday September 16, 2025

Subject: Short Note 4 – Accepted for publication

Dear Inès Achin and Elodie Chaillou,

We are pleased to inform you that your Short Note 4, entitled “Description of an experimental setup to investigate the impact of olfactory stimuli on behavioural responses in sheep (Ovis aries), with a particular focus on minimizing experimental bias through a habituation phase”, has been accepted for publication.

We thank you for carefully addressing the reviewers’ and editorial requests. Your efforts have improved the clarity, accessibility, and reporting of your work, thereby strengthening its reproducibility.

Your Short Note will shortly be assigned a DOI and made available on the Short Notes platform, together with the peer-review document annexed to the publication.

Congratulations, and thank you for contributing to robust, transparent, and reproducible science.

With best regards,
Marc Le Bert
Short Notes Editorial Team

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Final validation and publication: Tuesday September 16, 2025