Title: The use of pigs vocalisation structure to assess the quality of human-pig relationship

Dear authors,

I thank you for taking the comments from reviewers into account and for providing detailed explanations on how you handle them. There are still some comments that require further attention. See below text in red. I make some suggestions to further improve the manuscript. There are still few points that I would like to double check with Rev 2. I invite you to first take my suggestions into account before I liaise with Rev 2.

I thank you in advance.

**Letters to reviewers and Editor following Round #1**

Comment of Editor:

Status: Moderate revisions

This manuscript is of high interest. It however deserves amendments before we can recommend it. The two reviewers made detailed and complementary comments that should help the authors.

The writing needs to be improved to help the reading. The English needs also editing by a native english speaker.

Dear editor,

We now have made a revision of our manuscript. We answered all comments from both reviewers and the english was edited for the entire manuscript. We hope our changes will meet the reviewers’ expectations.

See below the detailed answers to each reviewer. All the best

Avelyne Villain and Céline Tallet, corresponding authors

**Reviews**

***Reviewed by Matteo Chincarini, 06 Apr 2022 07:44***

Dear Editor,

The manuscript “The use of pigs vocalisation structure to assess the quality of human-pig relationship” is addressing an original work on interspecific acoustic communication and explores the non-invasive emotional indicators in swine. The study design is very accurate, and the discussion of the results gives an exhaustive overview of the topic. A couple of concerns could be related to my misunderstanding. The first one regards the pen size where the experiment has been conducted and the second one is on the assumption test for PCA (please, see below). The experiment is well described and even if, working with farm animals and acoustic analysis is very challenging, the authors have worked very hard to set up elegant research. Furthermore, there is growing interest in vocal communication related to animal welfare as well as human-animal relationships. Finally, these results provide potential non-invasive indicators relevant to animal welfare. The manuscript needs also some minor revisions relative to the figures.

Below are some specific comments to the authors for minor revisions:

Dear reviewer,

Thank you for your review and your enthusiasm on the manuscript. We have addressed your comments and we hope our responses will clarify the concerns you had on the previous version of the manuscript. Please find below the detail answers (in blue) to your comments, with line referencing to the new version of the manuscript.

Title

The title clearly reflects the content of the article.

Abstract

The abstract is concise and presents the main findings of the study. I’ve only one concern regarding the first sentence: “In domestic species, studying human-animal interactions and their consequences on the establishment of a positive Human-Animal Relationship (HAR) would have applications for both improving animal welfare.” I’m not a native English speaker but here maybe it is possible to rephrase. I understand that authors are referring to animals and humans when they use the term “both” but now it seems it is referring to “interactions” and “consequences”.

The sentence has been changed. “Studying human-animal interactions in domestic species and how they affect the establishment of a positive Human-Animal Relationship (HAR) may help us improve animal welfare and better understand the evolution of interspecific interactions associated with the domestication process.“ line 18.

Introduction

Hypotheses have been explicit very clearly and they are supported by several papers representing the state of the art in this field.

Thank you for this comment.

LL 46-47: please consider merging these two very short sentences.

Former version “Domestic species form particular relationship with humans. In farms, this relationship is important for animal welfare.”.

This was changed to “In farms, the relationship that domestic animals form with humans is important for animal welfare” line 52

Materials and methods

This section is, in general, well explained and detailed. Thank you for this comment.

Ethical note

L 103: please, consider citing the French and European legislation (this will be relevant for the pen size, see below)

This text was added: “UE3P, where the experiment was carried out, is an experimental unit authorized by the French Ministry of Agriculture to breed animals for experimentation under the number D35-275-32. This authorization includes a derogation to follow the directive 2008/120/EC relative to the protection of pigs and its regulations.” line 113

Subject and housing conditions

L 112: please, verify the pen size according to your authorisation. According to the EU Directive 63/2010, the minimum enclosure size should be 2,0 m2 (Table 7.3). This could be not your case but it needs to be justified (it can be smaller due to experimental grounds).

You are right, but the experimental facilities have a derogation, as INRAE’s experimentation are done to develop application for livestock breeding. See response above and additional sentence in the manuscript “UE3P, where is experiments were carried out, is an experimental unit authorized by the French Ministry of Agriculture to breed animals for experimentation under the number D35-

275-32. This authorization includes a derogation to follow the directive 2008/120/EC relative to the protection of pigs and its regulations.” line 113

Conditioning

L 137: you say that conditioning took place between day 42 and 62 after weaning, so it would be between 70 and 90 days of life? At L 107 it is reported that piglets involved in the study were from 28 to 62 days of life, please double check it.

Thank you for this careful read, “day” refers days of life and this was made clear. This was a typo as the beginning of the experiment was after weaning (at 28 days of age).

“From day 28 (day of weaning) to day 39 of life, pigs were separated into two groups that experienced a different post-weaning period as follows:” See line 131

L 145: I’m not sure what “Hens” means here Multiples typos were corrected, it was one of them.

Behavioural monitoring and analysis

Please, specify if the behaviour has been analysed either by the same or different persons.

Yes, only one person scored the videos for behavioural analyses. See line 180 “ For every second trial, the two-minute reunions with the human were analysed by the same person : trials number 2, 4, 6, 8, 10 and 11”

Acoustic monitoring and analysis

L 178: Even if Praat is well-known software in this field, I think it would be better to cite it using a reference. Especially to be clear about the version that has been used (please, you can give a look here: https://[www.fon.hum.uva.nl/praat/manual/FAQ](http://www.fon.hum.uva.nl/praat/manual/FAQ) How\_to\_cite\_Praat.html)

Yes. See line 210 “Praat software (Boersma and Paul 2001), version 6.0 from [http://www.praat.org/.](http://www.praat.org/)“

Statistical analysis

I am not a statistician. However, when applying PCA I think it is worth reporting the value of Kaiser- Meyer Olkin (KMO) and Bartlett’s test of sphericity as preliminary tests (or explain why not). Maybe, you could also consider using the Measure of Sampling Adequacy (MSA) if some variable needs to be excluded.

From what we understood of the literature on the subject, Kaiser-Meyer-Olkin test is a measure of how the data are suited for Factor Analysis (<https://www.statisticshowto.com/kaiser-meyer-olkin/>). Although both a Factor Analysis and a Principal Component Analysis identify patterns and correlations between variables, they do not rely on the same assumptions. Contrary to the Factor Analysis, the mathematics behind the PCA does not assume the existence of latent factors underlying the observed data ([https://www.displayr.com/factor-analysis-and-principal-component-](https://www.displayr.com/factor-analysis-and-principal-component-analysis-a-simple-explanation/) [analysis-a-simple-explanation/](https://www.displayr.com/factor-analysis-and-principal-component-analysis-a-simple-explanation/)). In our case, the PCA is used to build composite scores (either composite behavioural scores or composite acoustic scores) to reduce the number of statistical variables (and avoid type I errors testing each variable one after the other). In addition, when measuring several vocal parameters, it happens indeed, that some of them are correlated and thus

load strongly together (see for example [Briefer et al 2019 on pig grunts](https://asa.scitation.org/doi/full/10.1121/1.5100612)). The PCA thus also allows us to visualize the parameters that load together on PCs and gives us a rationale for understanding the global acoustic structure of the calls (and not for clustering purposes for example). As a consequence, in our case, neither the Kaiser-Meyer-Olkin test nor the Bartlett’s test are necessary.

From Table 4, I’m understanding that you transformed some variables (like using log or sqrt). Please, consider adding this information also in the text when you write about symmetrical distribution L 206.

Yes, see line 245: “ linear transformations were computed when necessary to reach symmetrical distribution (see tables 2, 3, 4).”

Editor: It would be good to specify what (linear) transformations exactly were used

L 207: “pca”, did you mean function “dudi.pca”?

Yes, see line 248: “‘dudi.pca’ function from ‘ade4’ R package (Dray and Dufour 2007, 4)”

L 254: inside the code, I think ID/time/Phase should be ID/Time/Phase

Yes, see line 301 “Model2 <– lmer ( Vocal variable ~ Treatment \* Phase \* Time + Treatment \* HumanID + Time \* HumanID + Treatment \* Replicate + Time \* Replicate + (1 | PigID/Time/Phase) , data= dataVocalIsolation + dataVocalReunion).”

Results

General: most of the script has been reported, could you add also the PCA analysis? Regarding data availability:

* we have shared all datasets used in the study
* we have written a readme to guide readers through the dataset and explained which dataset corresponds to which analysis (<https://doi.org/10.15454/RTBO3O>).
* in the manuscript, we have made sure to report which R libraries and which functions in these libraries we used. All formulas of the statistical models are explicit in the text to facilitate transfer of information and replicate the analysis. All libraries are open source as well. See statement in the manuscript: “We have made sure to report in the main text of the article which R libraries and which functions in these libraries we used. All formulas of the statistical models are explicit in the text to facilitate transfer of information and replicate the analysis. All libraries are open source as well.” Line 741
* the PCAs were performed on raw parameters contained in the dataset we shared. All pre- processing transformations on parameters are reported in the manuscript. So the PCAs can also be redone from the datasets we shared.

This way anybody can redo any of the analysis represented in the paper. L 344: figure 3 is not present in the manuscript

The problem of figure referencing was solved.

L 369: figure 4, I’m not sure that is referring to the actual figure

The problem of figure referencing was solved. Tables and figures

Please, see above Corrected.

Discussion

The discussion is exhaustive and well supported by the literature. Still, the conclusions are not overstated.

Thank you for this comment. L 475: there are two “first” Corrected.

References Fine.

***Reviewed by anonymous reviewer, 04 May 2022 14:19***

Review of “The use of pigs vocalisation structure to assess the quality of human-pig relationship” by Villain et al.

This is an interesting study aiming to investigate if (changes in) pig vocalisation reflects the quality of human-animal relationships. I have, however, some major concerns and a few other issues, which I have summarised below.

Dear reviewer,

We thank you for your careful read of the manuscript and we apologize if the spelling made it difficult. We appreciated your comments and we did our best to answer to all of them, providing changes in the manuscript and adding tables as supplementary to meet your expectations.

Please find below the detail answer to your comments (in blue), with line referencing to the new version of the manuscript.

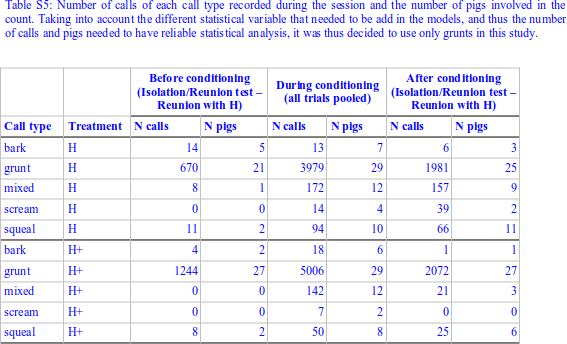
Major concerns:

The study sets out to analyse pig vocalisation in different situations, comparing changes in sound structure both within and between pigs when treated differently over time. However, the data are immediately reduced to only grunts (line 178) because they were the most frequent. Is frequency important for all calls? One scream may say more than a thousand grunts, to paraphrase Ibsen. I would like to see a couple of phrases explaining this a bit more, as this is an important aspect of your data editing. You also focus on vocal quality, but what about vocal quantity? You mention vocal activity in line97, but refer to some qualitative aspects there, too.

Editor: I will ask rev 2 to further check

We added a table in the supplementary material collecting the number of each call type per test and per treatment as well as the number of pigs involved in the count.

We hope this table will convince that regarding the number and the experimental design, an analysis of the quality of the vocalizations produced was not possible. Page 22 of the supplementary material and reference to this table in main text line 218.



I am missing which specific hypotheses you are testing? Or rather, in the Discussion, you dismiss one of the hypotheses, and then appear to suggest two new ones (lines 539-544).

In the paragraph before, we discuss the effect of the mere presence of a human on the structure of grunts, leading to two hypotheses. The aim was to announce these hypotheses and address them in the next paragraph “the interpretation of the second type of human-piglet interactions may allow to address these hypotheses” (in the version 1) but this was not clear enough.

We worked on the writing to increase clarity on that aspect. Lines XXX to line XXX: Beginning of discussion section:

* “In the next two paragraphs, we discuss the results of the standard reunion test before and after conditioning. This enables us to raise two possible hypotheses. We then use the results of the sessions of additional positive contacts of the conditioning to discuss theses hypotheses.” Line 583

End of paragraph of discussion of Isolation/Reunion test

“This test may allow us to suggest two potential non exclusive hypotheses to explain why the effect of human proximity on grunt acoustic structure attenuates as the familiarity to the human increases. In a first hypothesis, we could think that this attenuation of acoustic flexibility is due to a decrease in reactivity to the human, which may be linked to a disinterest of human contacts and an increase in foraging natural behaviours. In another hypothesis, this attenuation of acoustic flexibility may be due to a violation of piglets expectations: because the human remains static during the test, this may inhibit vocal reactions to the proximity. The interpretation of the second type of human-piglet interactions below may allow to address these hypotheses.” Line 638

Editor: I also think the discussion is not easy to follow, especially L588-679. The discussion would benefit from being shortened in length and focusing on the interpretation of grunts. Currently, you are discussing a lot the animal's behavior. In fact, the behavior helps you verify that your experimental design is adequate to produce different qualities of HAR and thenallows you to analyse how grunts vary with that quality. You could simplify the discussion of behavior (since that is not the focus of your paper), by avoiding going back and forth in your interpretation and rather offering an interpretation from the start that matches the responses observed both when the human is static or interacting. Then, the grunts should be analyzed with respect to the behavioral interpretations. These are present in your current discussion but are somewhat diluted in the behavioral discussion.

Was the vocalisation when conspecifics were social partners (line 141) used at all? I may have missed where that is presented. And if not, why was this included? And if used, how did you separate the vocalisation of the different pigs?

Vocalisations in relation to conspecifics arrival and the experimental design of the conditioning is already published [1](#_bookmark0). The reason why the two-way conditioning is explained in the method (with the human as the outcome and with the penmates as the outcome) is to be perfectly transparent on the full experiment and the different (pseudo)social experiences the experimental pigs were subjected to. The present article complete the preceding one on the same design, focusing on human-pig relationship.

Indeed, for ethical purposes, to limit the number of animals bred for experimental purposes, one experiment was designed with two (compatible) objectives. 1) Study the vocal and behavioural anticipation of (pseudo)social partners [using data of the conditioning before the reunion occurred, see Villain et al 2020, scientific reports]. 2) Study vocal and behavioural evolution of human-pig interactions [using data that were not explored in study 1]

See paragraph in the revised version of the manuscript Line 172: “Sessions of reunions with social partners were not studied and only served as reward during the conditioning in a previous analysis of vocal expression of positive anticipation (Villain et al. 2020).

Editor: the above sentence is not clear to me. It seems that you did study the sessions with social partners but not in this paper. I suggest : “Sessions of reunions with social partners are not studied here because they were part of an analysis on vocal expression of positive anticipationreported earlier (Villain et al. 2020)’

Indeed, first the two (pseudo) social contexts would have been difficult to compare (reunion between three pigs vs. reunion between one pig and one human). Second, regarding the vocal behaviour, the caller among the group of three pigs would not have been identified reliably, making it difficult to study within individual vocal flexibility”

The analyses are complex and can be difficult to follow in places. Is a p-value threshold of 0.05 too large for 3-way interactions? Some (many?) of your 3-way interactions have a p-value of 0.03 (even 0.07, which you still keep?), and I am left questioning how relevant they are. It leads to results like “grunts produced closer to the human were shorter… but only in untamed piglets, effect being stronger before the conditioning” and “grunts had a higher frequency range … when produced closer to the human…, but only in untamed piglets and before the conditioning”. On a data set of this size, I wonder to what extent these results can be generalised. It makes the manuscript very long and very difficult to follow in places – and the main results drown. Is there enough power to make such detailed conclusions?

This experiment has only a relatively complex design: two independent groups of pigs (H and H+) are subjected to a conditioning and their behaviour is studied before, during and after the conditioning: the purpose is clearly to study time effect in interaction with the treatment positive handling at weaning. However, several reports in the literature made us add some factors that we thought relevant regarding our question. Following previous reports of effects of the spatial proximity to a human on pig vocal behaviour, the ‘location’ of the pig also needed to be taken into account, especially because the proximity of the pig is a relevant descriptor of human-pig interactions. We thus used the three way interaction between “treatment\*conditioning time\*proximity” and the large sample size in the dataset allowed to do so.

Regarding your question on p value threshold, from our understanding of statistical models and testings, increasing the degree of interaction just makes the p-value threshold of 0.05 harder to reach but we don’t recall papers in the literature suggesting to change the significant threshold when dealing with three-way interactions. If we missed something, we would be happy to try a different model and/or a different significant threshold if suggested in the literature.

1 Villain et al., 2020 “Piglets Vocally Express the Anticipation of Pseudo-Social Contexts in Their Grunts.”

The outcome of the statistical results may thus look technical, but unfortunately, we think we must take the architecture of the experiment into account and that we must not oversimplify models and risk having conclusions beyond support.

Concerning the p-value between 0.05 and 0.1. Indeed, we did report one (problematic) three-way interaction: line 348 of the **version 1 of the manuscript** “For AcPC2, the three-way interaction was close to reach significant level (𝜒21 = 3.3, p = 0.07), thus, for conservative purposes, the results of the post hoc tests of the three-way interaction are presented (see two-way subsequent interactions comparisons in supplementary tables S2 and S3)”. Another sentence also explained this in the supplementary material “Note : due to a three-way interaction close to significance level, contrasts were generated with the three-way interaction and with the two-ways interactions of interests”.

Following your comments, we decided to keep the supplementary material as it was but to change the main text showing results of post hoc test on the two-way interactions (and not the tendency on the three-way interaction). However, we decided to keep a sentence of this three-way interaction to advertise the reader that this analysis also exists in the supplementary material if needed. The text has been changed

“For VocPC2, the three way interaction did not reach significance (𝜒2 = 3.3, p = 0.07), so only subsequent two way interactions were considered (but post hoc tests on the three way interaction can be found in supplementary, tables S1 to S3). For VocPC2, significant two way interactions were found between the conditioning time and the location (𝜒2 = 10.3, p = 0.001) on the one hand, and between the location and the treatment (𝜒2 = 4.2, p = 0.04) on the other hand. Post hoc tests revealed that grunts produced closer to the human had a higher VocPC2, meaning they had a higher pitch, effect being stronger before the conditioning than after (before: away – close, z.ratio = -6.12, p < 0.001; after: away – close, z.ratio = -2.88, p = 0.004, figure 3C). The increase in VocPC2 with the location was greater for non handled piglets than positively handled piglets (H piglets: away – close, z.ratio = -5.54, p < 0.001; H+ piglets: away – close, z.ratio = -3.82, p = 0.001, figure 3D). ” Line 412

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See also changes in figure 3, line 397.

Our results are in line with the literature on the effect of positive handling on behaviour and vocal quality, and our statistical approach reasonable, and thus their generalisation makes no real doubt to us. However, for sure it cannot be proven without any replicate of the design and will remain questionable.

Line 445-449: Is this what you expected? Could your interpretation be affected by the nature of the treatment, in other words, you will describe the response of the H+ pigs as positive (either being touch/attention satiated, or know they can always come back, and therefore disinterested, or confident enough to go exploring). How can this be disproven if you haven’t set out expected outcomes from the start?

Quote from version 1 of manuscript: “In addition, tamed piglets expressed more exploratory behaviours than untamed piglets after the conditioning (ReuPC3), which may be interpreted as natural foraging and disinterest from human contact, which may be a sign of positive welfare (Weerd and Day 2009). However, this could be interpreted also in terms of attachment to the human”

Expression of natural foraging has been hypothesized in previous literature on animal welfare and we cite this literature in the introduction “the decrease of experiencing negatively perceived contexts and the increase in experiencing positively perceived contexts and species-specific behaviors (Peterson, Simonsen, and Lawson 1995; Weerd and Day 2009).” Line 57.

In the discussion we thus confront our result of increased investigation of the room in more familiarised piglets (H+) to this literature, along with other marker of positive perception (attraction, contact to the human and time spent in proximity). All piglets at the end of the conditioning expressed behaviours in favour of an interpretation of positive perception of the human and H+ piglet expressed “something more”. We thus hypothesis later a sequential establishment of a positive HAR in piglets : attraction first and expression of foraging behaviour in a further “step” of relationship .

Paragraph of the discussion has been rephrased:

“This test also showed that the conditioning modified the behaviour of non handled piglets so that they finally expressed a similar attraction toward the human as positively handled piglets, after the conditioning.

Editor: consider rephrasing as ‘so that after the conditioning, they expressed a similar attraction toward the human as positively handled piglets’

These results are in line with the behavioural results of the sessions of additional positive contacts. The analysis of piglets’ behaviour every second sessions of the conditioning showed that, although positively handled and non handled piglets started with different degree of proximity toward the human (trials 2 and 4, CondPC1), then, over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human (CondPC1) and avoided less the human when the latter attempted to interact with them. At the end of the conditioning, piglets from both groups had similar level of proximity toward the human (trials 8, 10, 11 CondPC1)” Line 540

And:

“Beside behavioural proximity, piglets that were positively handled at weaning expressed more exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also observed during the sessions of additional positive contacts of the conditioning: positive handled piglets started with a higher score associated with investigation than non handled piglets (CondPC2) and it held over the conditioning. Piglets that were positively handled at weaning also expressed a higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an expression of natural foraging and disinterest from human contact, which may be a sign of positive welfare (Weerd and Day 2009). In addition, this could also be interpreted in terms of attachment to the human. Indeed, attachment to a human may facilitate exploration of novel environments or objects, as shown in dogs (Palmer and Custance 2008). A period of positive handling at weaning may provide an environment secure enough for the piglets to explore their environment in the presence of the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet, Veissier, and Boivin 2009). ” Line 556

And:

“We may be able to hypothesize a sequential establishment of a positive HAR over time: firstly with a decrease of attentive state and an increase in proximity and accepted contacts, and secondly with a disinterest of human contacts and the expression of natural foraging behaviour. The latter may require a higher exposure time.” Line 573

Editor: consider replacing ‘we may be able to hypothesize’ by ‘we hypothesize’

The last major concern is the text. The manuscript has a large number of typos (e.g. lines 405, 440, 463, 552 and elsewhere) and missing spaces, which leaves the impression that the uploading was rushed. I am usually quite forgiving when the English is a little bit rustic when the authors are writing in a second language. Unfortunately, there are places where it makes it difficult to understand what is meant (e.g. line 38: “a carrying human”; line 112: “on plastic duckboard and panels visually isolated pens”; line 568: “’AH’ was more entitled to trigger higher positive states”), and as a reviewer, it can be jarring to have to second-guess the content. I would therefore recommend that the revised manuscript is copy-edited by third party before re-submission.

The manuscript has been proof read by a native English speaker.

Other issues:

The use of the word ‘taming’ is confusing, as domestic pigs (Sus scrofa domesticus) are all tame compared to the wild boar (Sus scrofa). I suggest to change this to ‘positive handling’.

It was changed in the version 2 of the manuscript.

The authors refer to their study animals as piglets, but they are weaned and thus should be called pigs.

We agree it depends on the articles. Nevertheless, since one part of the experiment is already published (anticipation of rewards) and the term “piglets” has been accepted in the paper, we decided to keep piglet in this version to keep it homogeneous.

Line 19: objectify the quality?

Changed to “ Understanding and describing”. Line 21

Line 20: Is not all behaviour spatial?

Spatial behaviours, opposed to postural behaviours that could not track in the study (ear and tail posture, facial expression). Changed to “ social, spatial and postural behaviours” Line 22.

Line 25: replace breeding with husbandry Changed to “husbandry” Line 27.

Line 50: Animal welfare conveys? Consists of? Wording was changed.

Line 69 and elsewhere: associated with, not to This was corrected everywhere needed.

Line 88: What is ‘formant’?

Definition added in parenthesis. See line 94 “as well as higher formants (which are frequency peaks containing more energy than others)”

Line 113: metal chain? Yes.

Line 145 and elsewhere: Hence Yes.

Figure 1. Suggest using ‘area’ for the distal and proximal areas, to not confuse it with the 16 zones. It is not clear where the distal area is (above or below the dashed line).

It was clarified in the legend of the figure and the term “area” was used everywhere it was needed in the manuscript. Thank you.

Line 161: Where the sessions recorded or was the annotation done live?

Only videos were used. It was clarified. See line 193 “Sessions and tests were recorded using a camera (Bosh, Box 960H-CDD) and behaviours were scored *a posteriori* on videos using *The Observer XT 14.0* (Noldus, The Netherlands) software.”

Line 170: Replace over with other Yes. Done.

Table 1: “The number of times the piglet looked at other parts of the room” - as the pig will be looking somewhere at all times, this will always be within 1 of the previous variable (Nb looks toward human).

This code was to distinguish when the pig has the head down from when the pig has the head up but not watching the human (watching doors or walls). The description was changed to “The number of times the piglet looked at other parts of the room than the human or the floor (walls, doors)” Line 205.

Editor: could you simply say ‘The number of times the piglet looked at walls or doors’?

Table 2: The Table does not show “Behavioural response score for the reunion phase of the Isolation/Reunion test.” but “Percentage of explained variance and variable loadings of the principal component analysis for the first three PCs.”

Legend of table 2 and table 3 were rephrased

*“Table 2: Percentage of explained variance and relative loadings of parameters on PCs, following the Principal Component Analysis computed on the behaviours scored during the reunion of the Isolation/Reunion test. The first three PCs, having an eigenvalue above 1, constituted three behavioural scores: ReuPC1, ReuPC2, ReuPC3. Parameters that explain the most each PC are bolded (|loading|*

*>0.4).” Line 256*

*“Table 3: Percentage of explained variance and relative loadings of parameters on PCs, following the Principal Component Analysis computed on the behaviours scored during the sessions of additional positive contacts of the conditioning. The first three PCs, having an eigenvalue above 1 constituted three behavioural scores: CondPC1, CondPC2, CondPC3. Parameters that explain the most each PC are bolded (|loading|>0.4).” Line 262*

Table 2 and elsewhere: You use the word ‘parameter’ when ‘variable’ is the correct term.

In practice, parameters were used to build composite scores, used as response variables in statistical model. So, we tried to be consistent using the term “parameters” for specific measures (a behaviour or an acoustic parameter), the term “score” to refer to the PCs and the term “variable” for statistics. We doubled checked the consistency throughout the manuscript

Editor: could you explain that choice to the readers somewhere in the text?

Table 4: Are these variables on vocalisation characteristics essentially showing the same, leading to a high loading for all of them on PC1, i.e. so highly correlated that they are not all needed?

Yes, several parameters measured on vocalization, sometimes load on the same PCs and are sometimes highly correlated. It is one of the purpose of using a PCA, to quantify which parameters load together. Depending on the study, not the same parameters will load on PC1 for example,

sometimes parameters describing the noise components will load on a different PC than the one describing the frequency distribution (see Briefer at al 2019 on pig grunts for example).

Nevertheless, the PCA is used to have a non biased description of the structure of the vocalization, maximizing the variance, without having to pre select parameters.

Line 260: Vocal response variable See response above

Lines 323 and 326: Why are these estimates?

Since no figures of this specific analysis is presented in the main text to see the range of variation depending on contexts (not the main scope of the study but necessary as a control), we report the output of the model and thus the estimate and 95% confidence interval along with the statistics.

Figure 4: It is not possible to know what comparisons the letters refer to, as some of them have no letter. What is different from what? Question if a three-way interaction on a subset of data is biological relevant? The blue and grey colours are indistinguishable.

The contrast of the blue and grey scales was increased. We wrote “subset” of data to explain that the Isolation period of the test was not included in this analysis. Indeed, the isolation phase was just used as a negative control but the main scope was to analyse the sessions of reunion with the human. So the term subset may be misleading. The “subset” here constitutes an entire dataset of all grunts produced during the reunions with the human. The term was thus removed. See our response above regarding the the use of the three-way interaction. When no letters are used, it means that the groups does not significantly differ, a sentence was added in the legend.

*“Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect of conditioning (before or after), treatment (H or H+), and location of the pig relatively to the human (close: dark blue or away from them: light blue). Violin plots representing the median and the density of data distribution in the considered groups. (A, B) Results of post hoc tests following the significant three way interaction between the treatment, the conditioning time and the location on grunt duration (A) and on the first vocal score (-VocPC1, B). (C,D) Results of post hoc tests following the significant two way interactions between conditioning time and location*

1. *and between treatment and location (D) on the second vocal score (VocPC2). When involved in interaction, the conditioning time was fixed (as it was relevant to consider difference affected only by time). It thus allowed pairwise comparisons of interacting location and treatment (A, B) or levels of location (C). Letters represent significantly different groups (p < 0.05). When no letters are present, no significant difference between groups was found. Stars (\*) between two groups represent a statistical trend (p< 0.10). Full statistical report is available as supplementary material (tables S1 S2 for statistical test and S3 for model estimates).” Line 395.*

*Editor: The figure is confusing. The title is “effect of trial number” but on figures B and C you report difference between treatments. The fact that you don’t use the same code to report on significant results on all Fig is also a bit confusing. In addition, what are the z and y for in fig B? I suggest that*

* *you show also the results from the various trials on Fig 4B and C*
* *you add letters to show what is different from what*
* *you explain in the legend what time effects or treatment effects (or interactions) are significant*

Figure 5: What does H:N mean in the legend of A? Figure D is missing (referred to in Table heading). Not sure what C means - failed used in figure heading, missed used in y-axis label, but what does this show?

“N” was used to refer to the trial number and as the stars next to N was referring to the significant effect of Trial number. It was removed. The entire legend was updated.

*“Figure 5: Evolution of vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human ‘(close’: dark blue) or elsewhere in the room (‘away’ from the human: light blue) and treatment (H vs. H+ pigs) on grunt duration (A) and VocPC2 (B). (C) Mean ± SE per group, interacting effect of trial number and location of pigs on VocPC2. Different letters in A and B represent significantly different groups, ”\*” in C represents significant*

Editor: the proper wording is “values with no common letters differ significantly”. Indeed ‘ab’ has different letters than ‘a’ or ‘b’ but is not statistically different from them

I tend to have the same comment than for Fig 4, that is the same code (letters) should be used in Fig C, unless it gets very messy

*difference between the two slopes. Full statistical report is available as supplementary material (tables S1-S3).”* Line 457

Line 369: Here you refer to the wrong Figure. This was carried over for the rest of the manuscript, so that wrong Figures were indicated.

Yes, the problem has been solved.

Figure 6: Are these slopes based on linear regressions? is this justified?

On the figure, the raw data mean +/- se are indicated. Since the model was linear and the trial number was continuous, indeed, the estimates of the model are based on linear regressions (see slope estimates in statistical table S3 of the supplementary material)

Table 5: Does this not indicate either that vocal parameters are not very robust measures, or that you were unable to standardise your treatment?

We discuss that point in the discussion. These effects of the identity of the human needed to be reported and we thus suggest interpretations and future work in the discussion regarding this. Experimenters either failed mimicking each other (see line XXX, where it is specified they did their best)

“The experimenters tried to imitate each others behaviours (remote video monitoring) to decrease variability.” Line 147

" In our study, both humans that interacted with the pigs wear exactly the same clothes and standardized their tactile interactions toward the pigs before starting the study, and agreed on the rhythm and types of sounds (words, intonation) to use, to minimise generating variability although no systematic controls of the human behaviour or spectral feature of voices were performed here” line 697

Differences between the humans, like odors, may explain (like you suggest later and we added that point to the discussion as well.

“Our results show that the identity of the human may modulate piglet proximity and vocal behaviour but the design of this experiment does not allow to find the causes of these observations (behaviour, voice characteristics, or even odour profile).” Line 703

This could explain why the two experimenters had different effects on behavioural proximity of the pigs and vocal scores. An analysis of the experimenter‘s behaviour may add information to disentangle these points but we think it is out of the score of this paper and we suggest future work. Since both behavioural scores and vocal scores were affected (and not only vocal scores) we hypothesize that characteristics of the human may impact the effectiveness of positive handling, rather than the robustness of measures. But again, these effects bring new questions to the field.

Line 443: attraction instead of attractiveness Yes, corrected there and elsewhere.

Line 467-468: This is using your interpretation of the positively handled pigs to draw conclusions on the limitations on the control group. The set-up did allow them to explore.

We are not sure we understood your point. We are discussing the fact that one possible “model” in terms of establishment of HAR could be that first the fear is reduced and the attraction is increased but that the disinterest of human contact and natural behaviours may come later in the process.

Please see the new version of the paragraph to see if we understood your point.

We may be able to hypothesize a sequential establishment of a positive HAR over time: firstly with a decrease of attentive state and an increase of proximity and accepted contacts, and secondly with a

disinterest of human contacts and the expression of natural foraging behaviour. The latter may require a higher exposure time.

“We may be able to hypothesize a sequential establishment of a positive HAR over time: firstly with a decrease of attentive state and an increase in proximity and accepted contacts, and secondly with a disinterest of human contacts and the expression of natural foraging behaviour. The latter may require a higher exposure time.” Line 573

Editor: This conclusion is based on the observation of behavior. Does the analysis of grunts support it? What the relation between the changes in behavior and grunts?

Line 514: Double reference Yes, corrected.

Line 568: More likely to? But does this not show that this test or variable is not generalisable?

Not entirely, even if the identity of the human was in the model, the statistical analysis still found effects of the treatment / proximity / time. But it is true that we need more information to understand what are the causes of the effect of the human. We had a sentence on this point in the previous version (kept in the second).

“Thus, more studies of human features that are most likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In addition, studying human-piglet relationship in a more systematic way, as in other domestic species, for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies of interspecific relationships. However, the influence of human identity did not modify the general outcomes of our study, but only decreased some effects, suggesting that this variability does not modify the main results, but should be considered in future studies” Line 705

Line 577: The major difference to the pigs is more likely to be in the difference in smells of the two handlers. Was there any thought given to soaps and perfume? Even body odours differ.

See response above.

Lines 586-588: But some variables were not significant for one of the handlers.

It depends on the type of test and the variable. Only the first behavioural score ReuPC1 of the Isolation/Reunion showed a significant interaction between the humanID and the Treatment and it was not found at all for the sessions of additional positive contacts. Some vocal scores changed depending on the human but not in interaction with the treatment. We report these findings and discuss them to encourage the community to run more controlled experiments to test what is making different humans perceived differently, we are not claiming we have demonstrated any causes. As we said earlier, the fact that the identity of the human was in the full model means that this variability was in the model, and do not rule out other significant effects.

We added more information in the result section

* “During the reunions of the Isolation/Reunion test, the interaction between treatment and human identity was significant for the first behavioural proximity score (ReuPC1, 𝜒2 = 6.01, p = 0.01) but not the others (ReuPC2 and ReuPC3 (𝜒21 < 1.98, p > 0.16, table S1).” Line 496

1

* “These interacting effects of the human identity and treatment on behaviour were not found when considering the reunions of the conditioning (𝜒2 < 1.32, p > 0.25 for all CondPCs, table S1).” Line 501

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* “Interactions between the human identity and conditioning time were not significant, neither considering the reunions of the Isolation/Reunion test (ReuPCs, 𝜒21 < 0.642, p > 0.42, tables S1), neither the trial number during the session of additional positive contacts of the conditioning (CondPCs, 𝜒21 < 0.11 p > 0.74, table S1). ” Line 504

And in the discussion:

* “ The effect of the human did not interact with the conditioning time, leading to the conclusion that the difference between the two experimenter may have establishment during the period of positive handling at weaning, prior to the conditioning.” Line 684

Editor : please correct into The effect of the human did not interact with the conditioning time, leading to the conclusion that the difference between the two experimenters may have established during the period of positive handling at weaning, prior to the conditioning

Line 600: “We suggest that the use of vocalisations to assess quality of human-pig relationship could help to better monitor the parameters involved…” I don’t know what this means?

The sentence was changed. See line 725

“We suggest that analysing vocalisations structure may be a good tool to assess the quality of human-pig relationship and help monitor the establishment of a positive HAR.”

#### The use of pigs vocalisation structure to assess the quality of human-

* 1. **pig relationship**
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#### Key words:

1. Positive handling, Acoustic communication, Emotions, Mood, Behaviour, Welfare, Interspecific
2. interactions.

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#### Abstract:

1. Studying human-animal interactions in domestic species and how they affect the establishment of a
2. positive Human-Animal Relationship (HAR) may help us improve animal welfare and better
3. understand the evolution of interspecific interactions associated with the domestication process.
4. Understanding and describing the quality of an HAR requires information on several aspects of the
5. animal biology and emotional states (social, spatial and postural behaviours, physiological and
6. cognitive states). Growing evidence shows that acoustic features of animal vocalisations may be
7. indicators of emotional states. Here, we tested the hypothesis that the quality of vocal expression

25 may indicate the quality of HAR. At weaning, 30 piglets were positively handled by an

1. experimenter who talked to and physically interacted with them three times a day, while 30 other
2. piglets only received the contact necessary for proper husbandry. After two weeks, we recorded the
3. behaviours and vocalisations produced in the presence of the static experimenter for five minutes.
4. We repeated this test two weeks later, after a conditioning period during which human presence with
5. additional positive contact was used as a reward for all piglets. We hypothesized this conditioning
6. period would lead to a positive human-piglet relationship for all piglets. As expected, piglets that
7. were positively handled at weaning expressed a higher attraction toward the experimenter, and, after
8. the conditioning, piglets that were not positively handled at weaning expressed a similar level of
9. attraction than the positively handled ones. Piglets positively handled at weaning generally produced
10. shorter grunts than the other ones. However the latter expressed more flexibility in call structure
11. when vocalising close to a human, with a decrease of grunt duration and an increase in pitch,
12. frequency range and noisiness in their grunt. This differential effect of proximity between groups of
13. piglets was attenuated after the conditioning during a standard reunion with a static human but
14. remained over time when the human was providing additional positive contacts. Results suggest that
15. first, changes in vocal structure are consistent with indicators of positive states in the presence of a
16. human. Second, increasing familiarity and proximity between a human and a pig may induce
17. changes in the acoustic structure of its grunts. Third, a human providing additional positive contacts
18. triggers more changes in vocalisation structure than by their presence only. We show that
19. vocalisation structure may allow us to assess the quality of human-pig relationship.

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# Introduction

1. The process of domestication was conducted to shape physiology and morphology of domestic
2. animal species, but also their behaviour. It notably has shaped interspecific interactions between
3. human and non-human animals, by improving animals’ capacity to use human signals to adapt their
4. behaviour both decreasing fearfulness toward humans and increasing attention toward humans
5. (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is
6. important for animal welfare. Therefore, studying human-animal interactions and their consequences
7. to understand the mechanisms of emergence and maintenance of a positive human-animal
8. relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three
9. major aspects: the ability of an animal to control its mental and physiological stability (Broom,
10. 2011), the decrease of experiencing negatively perceived contexts and the increase in experiencing
11. positively perceived contexts and species-specific behaviors (Peterson et al., 1995; Weerd & Day,
12. 2009). A positive HAR is thought to be established through repeated positive interactions between
13. the human and the non-human animal. Some of the mechanisms involved in this process are:
14. accumulation of positive experiences through positive associative learning, modifications of
15. cognitive biases, shaping expectations from the non-human animal toward the human. A positive
16. HAR can be appreciated through behavioural and physiological measures, for example by assessing
17. the expression of positive emotions [reviewed in (Rault et al. 2020)]. Several behavioural measures
18. may help to define a positive HAR such as: short latency to approach and spatial proximity (Boivin
19. et al., 2000; Schmied et al., 2008), body postures (Villain et al., 2020b) or play behaviour
20. (Jerolmack, 2009). Contacts from a human such as stroking, may induce changes in body postures
21. and exposition of body areas by the animal to the human, supposedly vulnerable [central neck area in
22. cattle (Schmied et al. 2008), abdominal area in pigs (Rault et al., 2019)]. Such grooming solicitation
23. may be markers of engagement, trust and motivation to interact with the human. In most cases, these
24. behaviours are similar to those shown during intraspecific socio positive interactions, although there
25. are some species specific behaviours [e.g., dog vs.. wolf (Gácsi et al., 2005)]. Vocal behaviour may
26. also help defining the quality of an HAR. First, vocalisations are known to carry markers of the
27. emotional states in several bird and mammal species (Briefer, 2012, 2020). Markers of emotional
28. valence (positive versus negative) has been studied in domestic farm animals [reviewed in Laurijs et
29. al. (2021)]. Second, some vocalisations have been associated with positive interactions with humans,
30. for example the cat – human communication : purring is thought to be derived from mother pup
31. communication during nursing and is observed associated with care solicitation from humans;
32. meowing, which is not observed during intra specific interactions is thought to emerge from
33. associative learning during cat – human interactions (Brown & Bradshaw, 2014). This shows that
34. HAR may elicit specific vocalisations from the non human animal toward the human.
35. In pigs, diversified evidence attest the possibility of a positive HAR. Animals may be handled by
36. humans providing regular additional positive contacts, leading to the expression of a positive
37. perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests
38. showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et
39. al., 2015b). Pigs may recognise a human providing positive contacts compared to an unfamiliar one
40. and adapt their behaviour accordingly (Brajon et al., 2015c). Pigs may be sensitive to human voice
41. and respond accordingly (Bensoussan et al., 2019, 2020). Pigs vocalisations are diverse and linked to
42. their emotional states, attested by the use of positive or negative call types (Briefer et al., 2019, 2022;
43. Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related
44. to the valence of a situation or the arousal a situation may trigger for the animal. For example,
45. grunts, that are among of the most used vocal signals and various situations is now known to be a
46. flexible call: shorter grunts have been associated with positive situations (Briefer et al., 2019, 2022;
47. Friel et al., 2019), as well as higher formants (which are frequency peaks containing more energy
48. than others) and a lower fundamental frequency during positive situations (Briefer et al., 2019,
49. 2022). Grunt structure may also change according to the arousal of a situation, with a higher
50. frequency range and a higher bandwidth when the arousal increases (Linhart et al., 2015). In order to
51. determine if vocalisations may be used as non invasive indicators of the quality of human-pig
52. relationship by themselves, we tested whether they could encode the quality of the human-pig
53. relationship, through the vocal expression of emotional state. Because they are used in contexts of
54. different valence and arousal and in most pigs, we studied the spectro-temporal structure of grunts.
55. We predicted that if grunts carry information on the quality of the human-pig relationship, then 1. A
56. period of positive handling given by a human should modulate vocal quality of piglets when in
57. presence of the human, leading to grunts exhibiting markers of positive states (shorter grunts), 2.
58. spatial proximity toward the human should influence the spectro-temporal structure of grunts (higher
59. pitched grunts as the arousal increases). 107

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## Methods

### Ethical note

1. The study was approved by the ethic committee CREEA and received the authorization no.
2. APAFIS#17071-2018101016045373\_V3 from the French Ministry of Higher Education, Research
3. and Innovation. UE3P, where the experiment was carried out, is an experimental unit authorized by
4. the French Ministry of Agriculture to breed animals for experimentation under the number D35-275-
5. 32. This authorization includes a derogation to follow the directive 2008/120/EC relative to the
6. protection of piglets and its regulations. 117

### Subjects and housing conditions

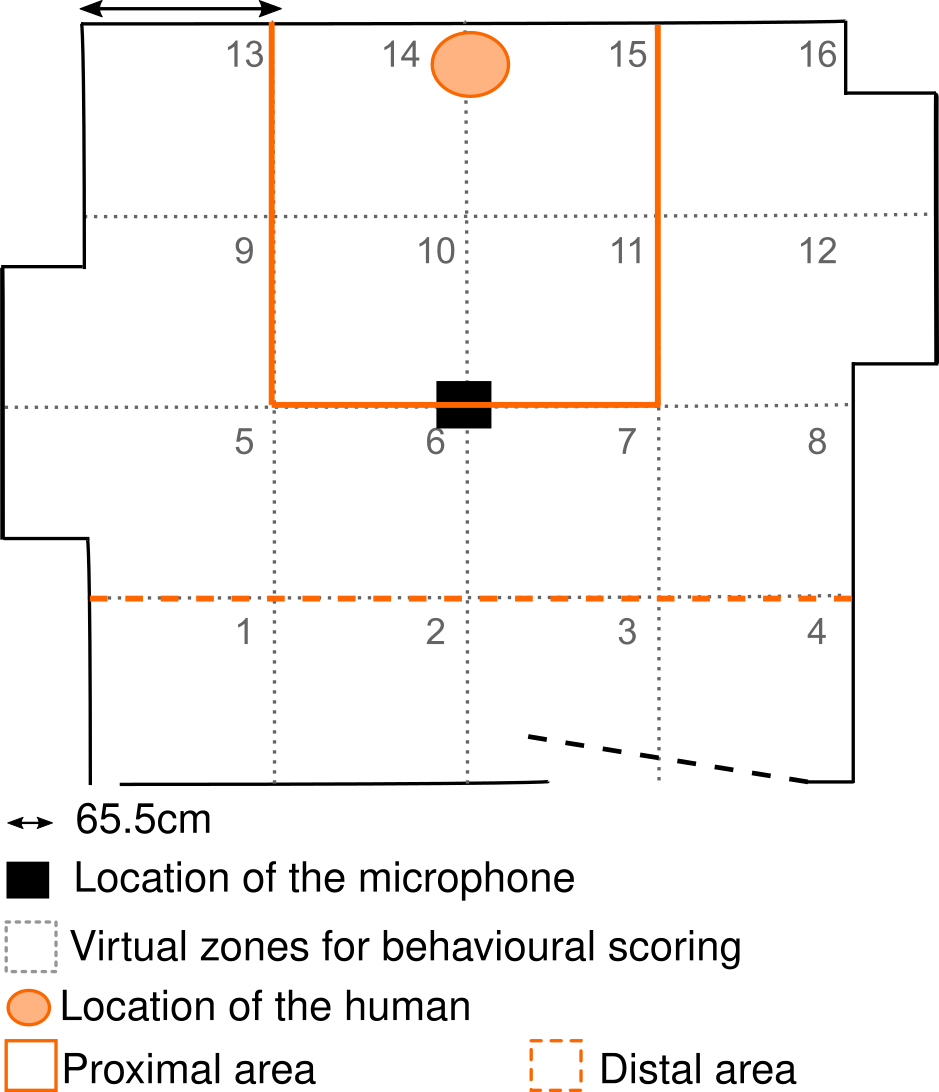
1. Sixty weaned female pigs (in two replicates from January to April 2019), *Sus scrofa domesticus*,
2. bred from crosses between Large White and Landrace females and Piétrain males were used for this
3. study from 28 to 62 days after birth. Animal housing and experiments took place at the experimental
4. unit UE3P (UE 1421, INRAE France).
5. One piglet had to be excluded from our sample size to receive care/medication due to health issues
6. independent from the experiment. From weaning at 28 days of age, piglets from the same litter and
7. having similar weight (<1 kg difference) were housed by three in a 1.2 x 1.3m pen on plastic
8. duckboard. Wooden panels were used to visually isolate pens. One metal chain per pen was used for
9. enrichment. Food and water were available *ad libitum*. Artificial lights were turned on from 8:00 to
10. 17:00 and temperature was maintained between 26 and 27 ºC. The experiment was carried out in two
11. replicates and two identical rearing rooms were used (5 pens per room per replicate).
12. ***Treatment: positive handling et weaning***
13. From day 28 (day of weaning) to day 39 of life, piglets were separated into two groups that
14. experienced a different post-weaning period as follows:
15. - Non positively handled piglets (H piglets): Control piglets from 10 rearing pens, housed in the
16. same room, received the minimal amount of daily contact with a stockperson (a 1.70m tall male who
17. did the feeding, cleaning and health checkups). The stockperson wore a dark green shirt and pants
18. and brown shoes.
19. - Positively handled piglets piglets (H+ piglets): Experimental piglets from the 10 other rearing pens,
20. housed in another room, received the same daily care given by the same stockperson as for H piglets.
21. They additionally received repeated sessions of additional human contacts. Each pen of three piglets
22. received 29 sessions of 10 minutes, from day 28 (weaning) until day 39, occurring five days a week.
23. Three sessions per day were performed (except on the day of weaning during which only two were
24. done with a two-hour break in between). Each session took place in the rearing pen and the order of
25. the interventions in the pens was balanced across days. The handling procedure, using gentle tactile
26. contacts is described in supplementary material of Villain et al. (2020) and was similar to Tallet et al.
27. (2014). Two experimenters performed these sessions (both women, both between 1.70-1.73 m tall,
28. with a balanced number of pens attributed to each of them). The experimenters wore the same blue
29. overalls and green boots each time they interacted with the piglets. The experimenters tried to imitate
30. each others behaviours (remote video monitoring) to decrease variability.
31. This intense period of additional positive contacts for half of the piglets after weaning constituted the
32. treatment of positive handling at weaning: positively handled piglets are referred to as H+ piglets and
33. non positively handled piglets are referred to as H piglets to describe the early experimental
34. treatment they experienced regarding a human, prior to the conditioning. 153

### Conditioning: sessions of additional positive contacts with (un)familiar human

1. The conditioning took place between day 42 and 62 of age and lasted twelve days, with two trials per
2. day and at least three hours between trials on the same day. Piglets were habituated to the test room
3. for 10 minutes, by pen, two days before the start of the conditioning. All piglets (H and H+) were
4. subjected to the same conditioning. The experimental design of the conditioning is already published
5. in an article dedicated to the study of anticipatory behaviour (Villain et al., 2020).
6. Briefly, all piglets were individually trained to learn to associate two different stimuli with the arrival
7. of two different (pseudo)-social partners: either two pen mates (partner = Conspecifics) or a familiar
8. human (partner = Human). When entering the room, the piglets and the partner(s) would remain in
9. the room for two minutes. Specifically, when the human was the partner, the human entered, sat on a
10. bucket and positively interacted with the piglet for two minutes, in the same manner as additional
11. contacts was provided to the H+ piglets during the previous period (see above section) (figure 1).
12. Therefore, at the beginning of the conditioning phase, H+ piglets were already familiar with the
13. human, whereas H piglets were unfamiliar with the human and only became familiar during the
14. conditioning.
15. The same sessions occurred in both treatment groups (H and H+). It was thus excepted that, at the
16. end of the conditioning, all piglets would be familiar with the human, but with a different degree in
17. H+ and H- piglets, due to a different time of exposure (H+: period of positive handling at weaning +
18. conditioning, H: conditioning only). Sessions of reunions with social partners were not studied and
19. only served as reward during the conditioning in a previous analysis of vocal expression of positive
20. anticipation (Villain et al., 2020). Indeed, first the two (pseudo) social contexts would have been
21. difficult to compare (reunion between three piglets vs. reunion between one piglet and one human).
22. Second, regarding the vocal behaviour, the caller among the group of three piglets would not have
23. been identified reliably, making it difficult to study within individual vocal flexibility.
24. For every second trial, the two-minute reunions with the human were analysed by the same person:
25. trials number 2, 4, 6, 8, 10 and 11 (see behavioural analyses section). 180

### Standard Isolation/Reunion Tests

1. At 40 or 41 (before conditioning) and then 63 or 64 (after conditioning) days of age, piglets were
2. subjected to a standard Isolation/Reunion test in order to assess their perception of the human. The
3. test consisted of two phases. The piglet was brought individually in a trolley to the experimental
4. room. It was left alone for five minutes, which defined the ‘Isolation’ phase. Then, the human
5. entered the room, remained stand up for 30 seconds and they sat on a bucket, remaining silent and
6. not moving for 4.5 minutes (figure 1).



1. *Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the*
2. *conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1,*
3. *2, 3, 4) were defined, suing the location of the human as reference.*

### Behavioural monitoring and analyses

1. Sessions and tests were recorded using a camera (Bosh, Box 960H-CDD) and behaviours were
2. scored *a posteriori* on videos using *The Observer XT 14.0* (Noldus, The Netherlands) software. The
3. room was split into 16 virtual equally-dimensioned zones to assess the mobility and exploratory
4. behaviour of the piglet. A proximal area, around the human was defined by merging four zones, a
5. distal area was defined merging the four most distant zones from the human (figure 1).
6. The behaviours scored during the reunion of the Isolation/Reunion test and the sessions of additional
7. positive contacts of the conditioning are available in table 1. Every time the shoulders of the piglet
8. crossed a zone, a zone change was scored. Looks and watching behaviours were scored as point
9. events, all other behaviours were scored as state events. Behavioural scores were then calculated to
10. quantify global responses (see below).
11. Table 1: Ethogram.

**Behaviour Description**

Nb zones crossed 1,2 The number of times the piglet crossed a virtual zone Nb approaches H 1 Number of times the piglets entered the proximal area Time watching H 1,2 The amount of time the piglet spent watching the human

Latency to contact H 1,2 The latency to the first contact of the human by the piglet

Nb looks toward H 1,2 The number of times the piglet turned its head toward the human

Nb looks other than H 1 The number of times the piglet looked at other parts of the room than the human or the floor (walls, doors)

Time watching room 1 The number of times the piglet watched other parts of the room than the human or the floor (walls, doors)

Time in proximal area 1,2 The amount of time the piglet spent in the proximal area Time in distal area 1,2 The amount of time the piglet spent in the distal area Time in contact H 1,2 The amount of time the piglet investigated the human Time investigating floor 1,2 The amount of time the piglet investigated the floor

Nb contacts H 2 Number of times the piglet was in contact with the human (initiated by the piglet or the human)

1: Scored during reunions of Isolation/Reunion tests. 2: Scored during reunions of conditioning sessions

1. 204

### Acoustic monitoring and analyses

1. Vocalisations were recorded with an AKG C314 microphone placed in the center of the room and
2. one meter above the ground, connected to a Marantz MD661MK2 recorder. Vocalisations produced
3. during each phase of the trial were manually annotated according to vocal type (grunt, squeal, bark,
4. scream and mixed calls (Kiley, 1972)), after visual inspection of spectrograms using the ‘Annotate’
5. function of the Praat software (Boersma & Paul, 2001), version 6.0 from [http://www.praat.org/.](http://www.praat.org/)
6. Checking the occurence of each call type in the several contexts of the study, we confirmed that
7. ‘grunt’ was the call type used in all contexts and by most of the piglets in each context. So only the
8. spectro-temporal structure of grunts was further analysed. For information, a table of the number of
9. each call types recorded in each context as well as the number of individuals involved in the count is
10. presented in the electronic supplementary material. We could not conduct a robust statistical analysis
11. on call type utterance, due to the rarity (per subject and tests) of other vocalisations than grunt. (table 217 S5).
12. A spectro-temporal analysis was performed with custom-written codes using the Seewave R package
13. (Sueur et al., 2008) implemented in R (R Core Team, 2015). We first studied the spectral properties
14. of the remaining background noise of the experimental room (electric noises and remaining low
15. frequency noises from the rest of the building), using 20 examples of 0.5 second fragments. Since the
16. first quartile (Q25) of the normalized spectrum of the background noise was 250Hz and the grunts
17. are low frequency vocalisations, we decided to remove all frequencies below 200Hz in order to focus
18. on the most relevant frequencies, using a 0.2-8 kHz bandpass filtering (‘fir’ function). As a
19. consequence, all results presented in this study are on a 0.2-8kHz frequency range, and no
20. conclusions on possible frequency components of grunts below this 200Hz threshold can be drawn
21. here. To measure grunt duration, a 5% to maximal amplitude threshold was used (‘timer’ function).
22. After normalisation, the following spectral parameters were calculated using the ‘specprop’ function
23. (FFT with Hamming window, window length = 512, overlap = 50%): mean (Q50), first (Q25) and
24. third (Q75) quartiles, interquartile range (IQR), centroid and standard deviation (all in Hz). The grunt
25. dominant frequency (in kHz) was also calculated (‘dfreq’, 50% overlapping FFTs, window length =
26. 512), which is the mean over the grunt duration of the frequencies of highest energy of each window.
27. Frequency peaks were detected and the minimal and maximal peaks were kept as descriptors
28. (‘fpeaks’ function, window length = 512, peak detection threshold = 10% of the normalized
29. amplitude). Measures of noisiness and entropy of the grunts were assessed using: Shannon entropy
30. (sh), Spectral Flatness (Wiener entropy, sfm) and Entropy (H) [combining both Shannon and
31. Temporal envelop entropy, length = 512, Hilbert envelop). Two vocal scores were used: the
32. logarithm of grunt duration and a built-in spectral vocal score with all spectral parameters (see
33. below). A table describing mean and range of variation of each acoustic parameter in the relevant
34. contexts of the study is available in the supplementary material (table S4). 241

### Statistical analyses

1. Behavioural and vocal response scores
2. The symmetrical distribution of all behavioural parameters on the one hand and all acoustic
3. parameters on the other hand was visually inspected, and linear transformations were computed
4. when necessary to reach symmetrical distribution (see tables 2, 3, 4). Two Principal Component
5. Analyses (PCA, one for the behavioural analysis and one for the spectral acoustic analysis) were
6. performed using all parameter having a symmetrical distribution (‘dudi.pca’ function from ‘ade4’ R
7. package (Dray & Dufour, 2007) and ‘inertia.dudi’ function to extract the loadings). Indeed, PCAs are
8. generally used to reduce the number of variables used in statistical models. It also generates
9. quantifiable global descriptors of behaviours or acoustic parameters, since correlated parameters
10. usually load on the same PC (McGregor, 1992). All PCs having an eigenvalue above one were kept
11. and constituted response scores of behavioural (‘ReuPCs’ and ‘CondPCs’ in table 2 and 3
12. respectively) and vocal (‘VocPCs’, table 4) parameters. Only the duration of grunts was kept
13. separated from the spectral parameters to keep it as a temporal parameter.
14. *Table 2: Percentage of explained variance and relative loadings of parameters on PCs, following the*
15. *Principal Component Analysis computed on the behaviours scored during the reunion of the*
16. *Isolation/Reunion test. The first three PCs, having an eigenvalue above 1, constituted three*
17. *behavioural scores: ReuPC1, ReuPC2, ReuPC3. Parameters that explain the most each PC are*
18. *bolded (|loading|>0.4).*

**ReuPC1 ReuPC2 ReuPC3**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Cumulative variance explained % | 38.3 | 60.8 | 74 |
| Nb of zones crossed | 24.177 | **-55.843** | -0.435 |
| Nb approaches H | **47.748** | -30.163 | 0.578 |
| Time watching H | **-52.914** | -7.422 | 25.585 |
| Latency to contact H | **-64.232** | -0.464 | 1.688 |
| Nb looks toward H | -7.787 | **-43.721** | 31.633 |
| Time watching room | -32.048 | -13.581 | -6.238 |
| Nb looks other than H | 3.524 | **-72.408** | -2.027 |
| Time in proximal area | **69.96** | -0.156 | 9.584 |
| Time in distal area | **-46.416** | -12.437 | -1.215 |
| Time in contact H | **61.041** | 3.586 | 24.183 |
| Time spent investigating floor | 11.868 | -7.503 | **-42.265** |
| 261 |  |  |  |  |

1. *Table 3: Percentage of explained variance and relative loadings of parameters on PCs, following the*
2. *Principal Component Analysis computed on the behaviours scored during the sessions of additional*
3. *positive contacts of the conditioning. The first three PCs, having an eigenvalue above 1 constituted*
4. *three behavioural scores: CondPC1, CondPC2, CondPC3. Parameters that explain the most each*
5. *PC are bolded (|loading|>0.4).*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Cumulative variance explained % | **CondPC1**  41 | **CondPC2**  68.5 | **CondPC3**  80.7 |
| Time in proximal area | **80.23** | 2.542 | -0.112 |
| Time in distal area | -33.826 | 8.547 | 30.789 |
| Number of contacts H | **78.55** | 6.476 | 2.288 |
| Time in contact H | **86.625** | 0.715 | -0.369 |
| Nb looks toward H | -2 | **79.898** | -0.745 |
| Time watching H | -6.757 | **65.67** | -10.325 |
| Nb of zones crossed | 0.129 | 33.599 | **48.457** |
| Time spent investigating floor | 0.006 | **-49.286** | 14.205 |
| 267 | Latency to contact H | **-81.01** | -0.248 | -2.83 |

1. *Table 4: Percentage of explained variance and relative loadings of parameters on PCs following a*
2. *Principal Component Analysis on spectral parameters of the grunts recorded in the entire dataset*
3. *(including both types of tests, N=17 546 grunts). The transformations used to reach symmetrical*
4. *distribution before the PCA are indicated in parentheses. The first three PCs, having an eigenvalue*
5. *above 1 constituted three vocal response scores: VocPC1, VocPC2, VocPC3. Parameters that*
6. *explain the most each PC are bolded (|loading|>0.4) .*

**VocPC1 VocPC2 VocPC3**

|  |  |  |
| --- | --- | --- |
| Cumulative variance explained % 59.769 | 76.807 | 87.712 |
| Mean Dominant Frequency1  -13.558 | **53.557** | 2.220 |
| Min frequency peak1 (log)  -0.349 | **58.758** | 24.236 |
| Max frequency peak1  **-43.023** | 8.760 | -9.537 |
| Mode2 (log)  -0.522 | **66.248** | 19.268 |
| Mean2 (log)  **-95.092** | -2.295 | 2.028 |
| Q502 (log)  **-85.278** | 0.280 | -0.093 |
| Q252 (log)  **-52.360** | 19.327 | 0.985 |
| Q752 (sqrt)  **-88.925** | -4.645 | 2.309 |
| Centroid2 (log)  **-95.092** | -2.295 | 2.028 |
| Sd2  **-64.484** | -11.303 | 7.680 |
| IQR2  **-87.981** | -5.851 | 2.640 |
| Sfm3 (sqrt)  **-94.344** | -3.189 | 0.962 |
| Sh3 (sqrt)  **-96.087** | -0.785 | -0.175 |
| H3  **-88.205** | -1.059 | -1.063 |
| Skewness4  28.032 | -18.010 | **48.652** |
| Kurtosis4  22.973 | -16.241 | **50.615** |

1: parameters related to the pitch of the vocalisation; 2: parameters related to the frequency distribution descriptors; 3: parameters related to the noise component of the vocalisation; 4: parameters related to the shape of the frequency distribution

1. Statistical models
2. All statistics were carried out on R (R Core Team, 2015). Linear mixed effect models (‘lmer’
3. function, ‘lme4’ R package (Bates et al., 2014)) were built when studied variables were linear
4. (behavioural and vocal scores, grunt duration) and one binomial generalized mixed effect model was
5. built for binary parameters (occurrence of missed contacts initiated by human during the
6. conditioning). The following subsections describe how models were built for each type of tests. In all
7. models described below, the identity of the replicate (‘1’ or ‘2’) was used as an interacting fixed
8. factor, since the experiment was run in two identical replicates on two independent groups. The
9. identity of the human (‘AH’ or ‘AV’) was used as interacting fixed factor in all models described
10. below, since two experimenters were involved in the positive handling at weaning and in the session
11. of additional positive contacts of the conditioning (but always the same human was attributed to a
12. given piglet). The piglet was used as random factor to take into account the within-subject design.
13. *Isolation/Reunion tests*
14. The aim of this part was to test the effect of the positive handling at weaning treatment (H vs. H+
15. piglets) and additional human contacts during sessions of the conditioning on the piglet’s reaction to
16. human presence. Since the same Isolation/Reunion test was repeated before and after the
17. conditioning, we used the variable ‘Conditioning time’ as a two level interacting factor (‘before’ or
18. ‘after’ the conditioning) to test the effect of the conditioning.
19. Model1 <– lmer (Response variable ~ Treatment\*Time + Treatment\*Replicate +
20. Treatment\*HumanID + Time\*Replicate + Time\*HumanID + (1 | pigletID), data=
21. dataBehaviourReunion).
22. Concerning the analysis of vocal behaviour, the isolation phase represents a negative social context
23. for the piglets and may be used as a negative control when monitoring the effect of human presence
24. on vocal expression of emotional states (Villain et al. 2020a). So, the two phases of the test were
25. used to study the three way interaction between the treatment (H vs.. H+), the phase of the test
26. (isolation vs.. reunion) and the time of the conditioning (before vs.. after). The following model was
27. computed :
28. Model2 <– lmer (Vocal response score ~ Treatment\*Phase\*Time + Treatment\*HumanID

302 + Time\*HumanID + Treatment\*Replicate + Time\*Replicate + (1 |

1. pigletID/Time/Phase) , data= dataVocalIsolation + dataVocalReunion).
2. To go further, only the reunion phase was kept and a proximity variable was added. Indeed, the
3. piglet could vocalise either when near the human or away from them and this spatial proximity was
4. demonstrated as an important factor of changes of vocal features (Villain et al. 2020b). Thus, a two
5. level proximity factor was built: either ‘1’ when the piglet was in the proximal area (figure 1) or ‘0’
6. when it was elsewhere in the room.
7. Model3 <- lmer (Vocal response score ~ Treatment\*Time\*InProxArea +
8. Treatment\*HumanID + InProxArea\*HumanID + Treatment\*Replicate +
9. InProxArea\*Replicate + Time\*Replicate + Time\*HumanID + (1 | pigletID/Time), data

312 = dataVocalReunion).

1. *Conditioning trials*
2. The aim was to study the evolution of human-piglet relationship over the conditioning [the variable
3. ‘Trial number’, used as a continuous variable], depending on the previous experience piglets had
4. with the human [either already familiar (H+ group) or unfamiliar (H group) at the beginning of the
5. conditioning]. Trial number (Trial) was also used as a random slope to take into account individual
6. trajectories (Schielzeth and Forstmeier 2009). The following model was built to test the behavioural
7. response scores (lmer) and the occurrence of missed contact initiated by the human during a session
8. (presence/absence, binomial model, glmer):
9. Model4 <– (g)lmer (Behavioural Response score ~ Trial\*Treatment + Trial\*HumanID

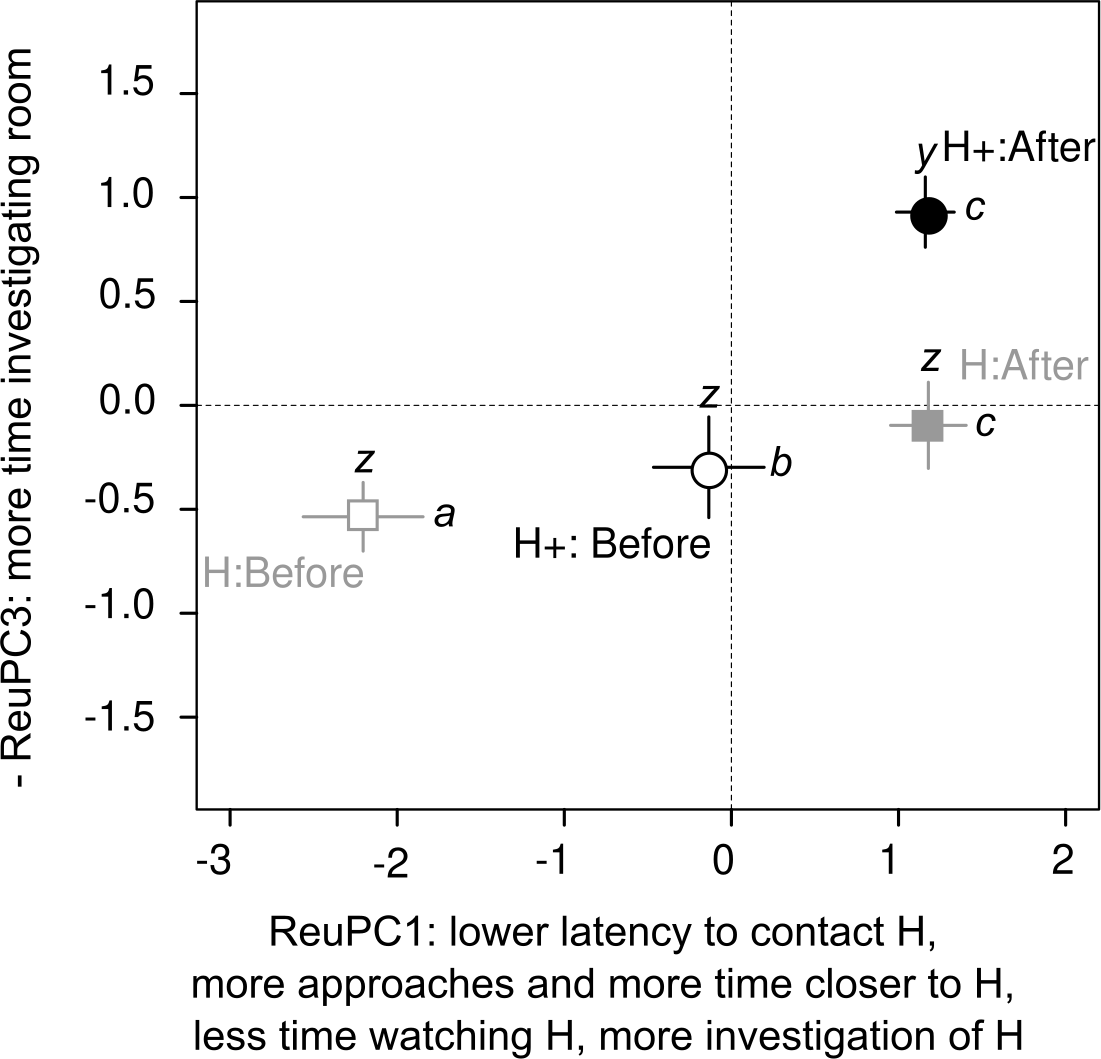
322 + Trial\*Replicate + Treatment\*Replicate + Treatment\* umanID + (1+ Trial |

1. pigletID), (family=Binomial), data= dataBehaviourConditioning).
2. For the analysis of vocal response scores, similarly to the Isolation/Reunion test, the piglet could
3. vocalise either when near the human or away from them. We thus added the proximity factor in the
4. analysis of vocal response variables. The following model was built :
5. Model5 <– lmer (Vocal Response score ~ Trial\*Treatment\*InProxArea+
6. Trial\*HumanID + Trial\*Replicate + Treatment\*Replicate + Treatment\*HumanID +
7. HumanID\*InProxArea + Replicate\*InProxArea + (1+ Trial | pigletID), data=
8. dataVocalConditioning).
9. *Model validation and statistical tests*
10. All linear models were validated by visual inspection of the symmetrical and normal distribution of
11. the residuals. Anovas (‘car’ R package (Fox & Weisberg, 2011)) were computed on models to test
12. for significant effects of explanatory variables. Following the Anova, when interactions were found
13. significant, post hoc test were run on model interactions, correcting for multiple testing with Tukey
14. contrasts (‘emmeans’ or ‘lstrends’ functions from ‘emmeans’ R package (Lenth, 2016), for
15. categorical or continuous variables respectively). Results of the Anova, model estimates and pairwise
16. post hoc comparisons are reported in the supplementary material (tables S1 and S2 for tests, table S3
17. for model estimates). 341

## Results

### Effect of the conditioning process on piglets’ reaction to human presence

1. ***(Isolation/Reunion tests)***
2. *Piglets that were not positively handled at weaning express a similar behavioural*
3. *proximity to a human after a positive conditioning as positively handled ones.*
4. 347



1. *Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human*
2. *during the reunion of the Isolation/Reunion test. Mean ± SE per group is indicated, different letters*
3. *indicates significantly different groups. Significant interaction between treatment (H : grey squares*
4. *and H+ : black circles) and time (Before the conditioning: empty elements and After the*
5. *conditioning: filled elements) on behavioural PC1 (letters a to c) and PC3 (letters z and y). Full*
6. *statistical report is available as supplementary material (tables S1 S2 for statistical tests and S3 for*
7. *model estimates)*
8. The interaction between the treatment (positively handled piglets before the conditioning (H+) or not

356

(H)) and the conditioning time (before or after the conditioning) was significant for both ReuPC1

1. and ReuPC3 (𝜒2 = 28.0, p < 0.001, and 𝜒2 = 3.7, p = 0.05 respectively, figure 2) but not for ReuPC2

1

1

1. (𝜒2 < 0.001, p = 0. 99, supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was

1

1. higher after the conditioning than before (H: after – before, t.ratio = 12.1, p <0.001 , H+: after –
2. before t.ratio = 11.0, p < 0.001) and that before the conditioning, piglets that were positively handled
3. at weaning had significantly higher ReuPC1 than non handled piglets (Before, H – H+: t.ratio = -2.1,
4. p < 0.001), but not after (After, H – H+: t.ratio = 0.02, p = 1.0). According to the loadings, this
5. means that piglets that were positively handled at weaning had a lower latency to contact the human,
6. approached them more often and spent more time close to and investigating the human (ReuPC1)
7. than non handled piglets, before the conditioning. This score increased after the conditioning and no
8. evidence of a difference between treatments after the conditioning was found (figure 2). Post hoc
9. tests on ReuPC3 showed a significant effect of the conditioning time only in piglets that were
10. positively handled at weaning (H+: after – before, t.ratio = 5.2, p < 0.001, H: after – before, t.ratio =
11. 2.6, p = 0.06). No difference in ReuPC3 was found between treatments before the conditioning
12. (Before: H – H+, t.ratio = -0.75, p = 0.87), whereas positively handled piglets had a higher -ReuPC3
13. after the conditioning than before (After : H – H+, t.ratio = -3.2, p = 0.009). According to the
14. loadings, this means that piglets that were positively handled at weaning expressed more
15. investigation of the room after the conditioning than before. No evidence of any effect on ReuPC2
16. was found (table S2).
17. *Positive handling at weaning decreases grunt duration even when no human is present*
18. *with the piglet*
19. Comparing the effect of the phase of the test (Isolation vs.. Reunion with the human), taking into

conditioning time:phase, conditioning time: treatment interactions : 𝜒2

378

account the conditioning time and the treatment, no evidence of any effect of neither the three way

379 interaction (𝜒2 < 0.62, p > 0.43) nor two way interactions of interest was found (treatment: phase,

380

<3.5, p > 0.06, table S2) in

382

1

produced by non handled piglets (𝜒2

1

381 any of the scores. However, grunts produced by positively handled piglets were shorter than grunts

1 = 5.5, p = 0.02, estimates of log(duration)[95% confidence

𝜒

1. interval]: -1.25[-1.32;-1.19] and -1.12[-1.2;-1.1] respectively in H+ and H piglets, table S3). Single
2. effects of the phase of the test were significant for grunt duration and all AcPCs ( 2 > 6.6, p < 0.01,

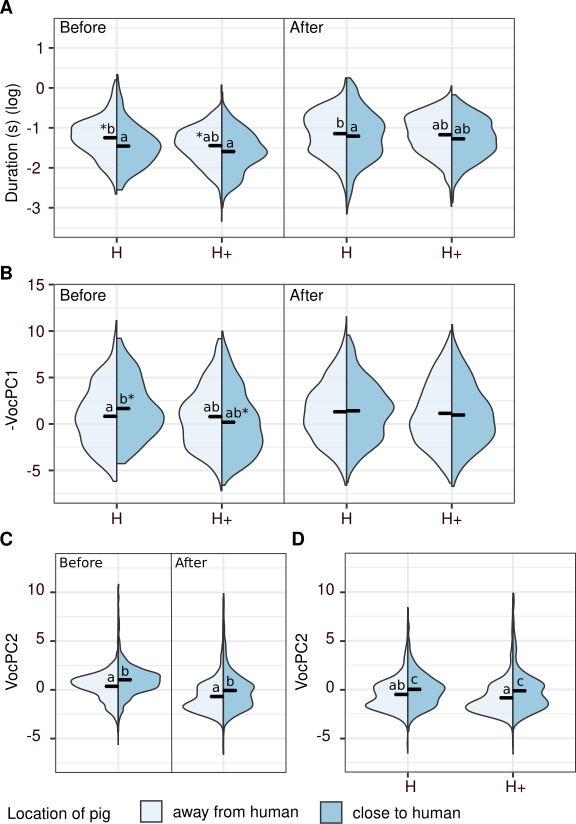
1

1. table S1). Grunts produced during the reunion phase with the human were shorter (estimates of
2. log(duration) : -1.32[-1.37;-1.26] vs.. -1.06[-1.12;-1.00]) and, according to the loadings, grunts
3. produced during the reunion phase had a higher frequency range, higher bandwidth and a higher 388 noise component (-VocPC1: 0.78[0.48;1.08] vs.. 0.34[0.03;0.66]), higher pitched (VocPC2: -0.18[-

389 0.36;0.01] vs.. -0.46[-0.65;-0.28] ) and their spectrum had a higher skewness and kurtosis (VocPC3: 390 -0.25[-0.37;-0.14] vs.. -0.11[-0.23;0.01] ), compared to the isolation phase.

391

1. *The conditioning process attenuates the effect of proximity on grunts vocal features in*
2. *non handled piglets*
3. 394



1. *Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect*
2. *of conditioning (before or after), treatment (H or H+), and location of the piglet relatively to the human (close: dark blue*
3. *or away from them: light blue). Violin plots representing the median and the density of data distribution in the*
4. *considered groups. (A, B) Results of post hoc tests following the significant three way interaction between the treatment,*
5. *the conditioning time and the location on grunt duration (A) and on the first vocal score (-VocPC1, B). (C,D) Results of*
6. *post hoc tests following the significant two way interactions between conditioning time and location (C) and between*
7. *treatment and location (D) on the second vocal score (VocPC2). When involved in interaction, the conditioning time was*
8. *fixed (as it was relevant to consider difference affected only by time). It thus allowed pairwise comparisons of interacting*
9. *location and treatment (A, B) or levels of location (C). Letters represent significantly different groups (p < 0.05). When*
10. *no letters are present, no significant difference between groups was found. Stars (\*) between two groups represent a*
11. *statistical trend (p< 0.10). Full statistical report is available as supplementary material (tables S1 S2 for statistical test*
12. *and S3 for model estimates).*
13. During the five-minute reunion, the piglet was scored either as close to the human or away from

408

them. The three way interaction of the conditioning time, the treatment and the location was

1. significant for grunt duration, -VocPC1 and VocPC3 (𝜒2 > 4.9, p < 0.03). Post hoc tests revealed

1

1. that grunts produced closer to the human were shorter than the ones produced further away, but only
2. in piglets that were not positively handled at weaning, effect being stronger before the conditioning
3. than after it (H piglets: away – close, z.ratio = 6.3, p < 0.001 before and z.ratio = 4.1 p < 0.001 after
4. the conditioning; H+ piglets: away – close z.ratio < 1.98 p > 0.19, figure 3A). -VocPC1 was higher,
5. i.e. grunts had a higher frequency range, bandwidth and were noisier when produced closer to the
6. human than further away, but only in non handled piglets and before the conditioning (H piglets:
7. away – close, z.ratio = -3.34, p = 0.005 before and z.ratio = -1.23 p = 0.61 after the conditioning; H+

1

considered (but post hoc tests on the three way interaction can be found in supplementary, tables S1

417

piglets: away – close, z.ratio < 0.36 p > 0.21, figure 3B). For VocPC2, the three way interaction did

418 not reach significance (𝜒2 = 3.3, p = 0.07), so only subsequent two way interactions were

419

420

to S3). For VocPC2, significant two way interactions were found between the conditioning time and

421 the location (𝜒2 = 10.3, p = 0.001) on the one hand, and between the location and the treatment (𝜒2

1

1

422 = 4.2, p = 0.04) on the other hand. Post hoc tests revealed that grunts produced closer to the human

1. had a higher VocPC2, meaning they had a higher pitch, effect being stronger before the conditioning
2. than after (before: away – close, z.ratio = -6.12, p < 0.001; after: away – close, z.ratio = -2.88, p =
3. 0.004, figure 3C). The increase in VocPC2 with the location was greater for non handled piglets than
4. positively handled piglets (H piglets: away – close, z.ratio = -5.54, p < 0.001; H+ piglets: away –

𝜒

1. close, z.ratio = -3.82, p = 0.001, figure 3D). The last two-way interaction of interest between the
2. conditioning time and the treatment did not reach significant level ( 2 = 0.80, p = 0.37). For

1

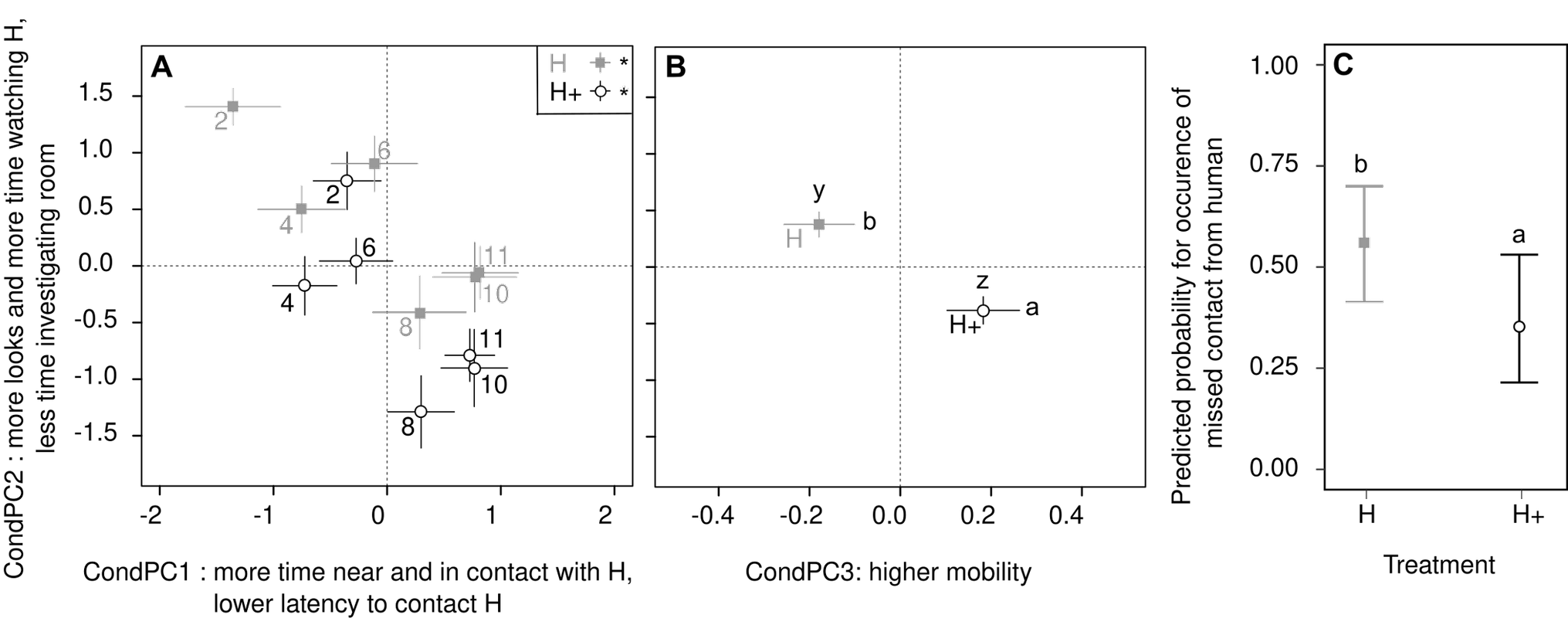
1. VocPC3, post hoc tests did not reach significant levels (|z.ratio| < 2.3 p > 0.09 for any comparison) . 430

### 431 Emergence of positive perception of human (effect of additional positive contacts sessions

432 ***over the conditioning)***

433 *The conditioning process increases behavioural proximity*

434



1. *Figure 4: Effect of trial number over the conditioning on spatial behaviour and proximity to the human during the 2min*
2. *sessions of additional positive contacts of the conditioning. (A, B) Mean ± SE per group. (A) Single effect of trial number*
3. *on behavioural scores CondPC1 and condPC2 according to treatment (H: grey, H+: black). (B) Single effect of*
4. *treatment on behavioural score CondPC3 and CondPC2. (C) Single effect of treatment on predicted probability of*
5. *occurrence of at least one missed contact by the human, mean estimates ± 95% confidence interval from the generalized*
6. *mixed effect model. Stars in the legend box represent significant effect of the trial number (A), different letters represent*
7. *significantly different groups (B and C). Full statistical report is available as supplementary material (tables S1 et S2 for*
8. *statistical tests, table S3 for model estimates).*
9. No evidence of any effect of the interaction between the treatment [positively handled piglets before
10. the conditioning (H+) or not (H)] and the trial number was found for all behavioural scores

𝜒

1. (CondPC1, CondPC2 and CondPC3, table 3). Independently from the treatment, the higher the trial
2. number the higher CondPC1 ( 2

1 = 59.3, p < 0.001, slope estimate [95% confidence interval]: 0.20

448 [0.15 : 0.25]) and the lower CondPC2 was (𝜒2 = 48.6, p < 0.001, slope estimate: -0.17 [-0.22 : -

1

449 0.12]). According to the loadings, over the conditioning, piglets decreased the latency to contact the

450 human, made more contacts, spent more time in the proximal area and in contact with the human

451 (condPC1), decreased the number of looks to the human, spent less watching the human and more

453 handled piglets had a lower CondPC2 and a higher CondPC3 than the non handled ones (𝜒2 = 12.8,

452 time investigating the room (CondPC2) (figure 4A). Independently from the trial number, positively

454 p < 0.001 and 𝜒2

1

1 = 7.0, p = 0.008 respectively), meaning that piglets that were positively handled at

455 weaning expressed a fewer number of looks to the human, spent less time watching them and more

456 time investigating the room (CondPC2) and crossed more virtual zone during the test (CondPC3)

𝜒

457 (figure 4B). The probability of having at least one missed contact by the human during a session was

458 lower for positively handled piglets than non handled ones ( 2

459

interaction with the trial number (𝜒2

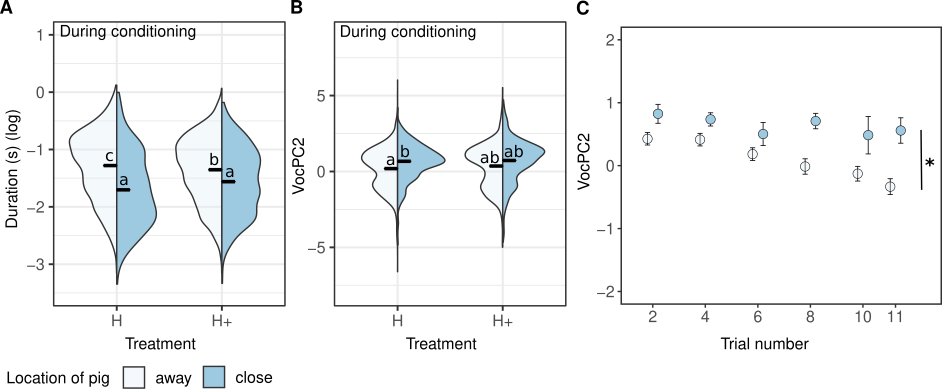
= 0.22, p = 0.064).

1 = 9.57, p = 0.002, figure 4C), with no

1

460

1. *Grunt acoustic features depends on spatial proximity to human*



1. *Figure 5: Evolution of vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B)*
2. *Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in*
3. *proximal area of the human ‘(close’: dark blue) or elsewhere in the room (‘away’ from the human: light blue) and*
4. *treatment (H vs. H+ piglets) on grunt duration (A) and VocPC2 (B). (C) Mean ± SE per group, interacting effect of trial*
5. *number and location of piglets on VocPC2. Different letters in A and B represent significantly different groups, ”\*” in C*
6. *represents significant difference between the two slopes. Full statistical report is available as supplementary material*
7. *(tables S1-S3).*
8. During the sessions of additional positive contacts of the conditioning, the three-way interaction

scores (𝜒21 < 0.18, p > 0.67), allowing the analysis of the two way interactions of intere2 st. The

1. between the trial number, the treatment and the location was not significant for any of the vocal 472

𝜒

1. interaction between treatment and the trial number was not significant for all vocal scores ( 1 < 2.5 p

𝜒2

1. > 0.11), however grunt duration decreased over the conditioning sessions (trial number:replicate
2. interaction, <5.3 p = 0.02, slope estimate -0.03[-0.04;-0.01] for the lower slope, table S1 and S3).

1

1. Independently from the trial number, grunt duration was lower when piglets were located close to the

𝜒2

1. human and this effect was stronger in non handled piglets than positively handled piglets
2. (treatment:location interaction: 1 = 15.8 p < 0.001, away vs.. close, H piglets: z.ratio = 10.2 p <

𝜒2

1. 0.001, H+ piglets: z.ratio = 6.86 p < 0.001, figure 5A). VocPC2 was higher when piglets were close
2. to the human, but only in non handled piglets (treatment:location interaction, 1

= 7.6 p = 0.005,

1. pairwise comparisons away vs. close, in H: z.ratio = -4.9 p z 0.001 and in H+: z.ratio = -2.0 p =
2. 0.21), meaning that non handled piglets produced higher pitched grunts when closer to the human

483

485

(figure 5B). The effect of the location on -VocPC1 and VocPC2 depended on the trial number (trial

= 3.97 p = 0.05 and

VocPC2 were higher when closer to the human with a greater extent later in the conditioning than

484 number : location interaction, 𝜒21 𝜒21 = 6.1 p = 0.01 respectively): -VocPC1 and

486 earlier (slope comparison away – close, -VocPC1 : z.ratio = -1.80 p = 0.07, VocPC2 : z.ratio = -2.34

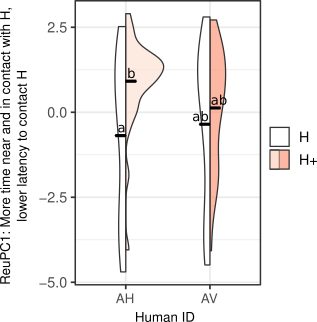
487 p = 0.02). According to the loadings, this means that the frequency range, bandwidth and noisiness of

488 grunts (-VocPC1) as well as the pitch (VocPC2) decreased over the conditioning when piglets were

489 located away from the human but remained high when piglets were close (figure 5C and 5D). 490

491 ***Impact of human identity on piglets behaviour and grunt structure***

492



1. *Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test.*
2. *Violin plots representing the median and the density of data distribution in the group. Different letters represent*
3. *significantly different groups. Full statistical report is available as supplementary material (tables S1 and S2 for*
4. *statistical tests, table S3 for model estimates).*
5. Since half of the piglets had been assigned to one human experimenter and the other half to another
6. one, the identity of the human was included in the model. This allowed to test interactions between
7. the identity of the human and the treatment of positive handling at weaning on the one hand and the
8. conditioning time on the other hand.

501

During the reunions of the Isolation/Reunion test, the interaction between treatment and human

502 identity was significant for the first behavioural proximity score (ReuPC1, 𝜒2 = 6.01, p = 0.01) but

503

1

504 ReuPC1 was higher when piglets were handled by the human ‘AH’ (H vs. H+, AH: t.ratio = -4.77, p

not the others (ReuPC2 and ReuPC3 (𝜒2

1

< 1.98, p > 0.16, table S1). The effect of treatment on

505 < 0.001, figure 6). When the human ‘AV’ handled the piglets, for which ReuPC1 scores exhibited

506 intermediate values, treatment was not significant (AV, H vs. H+: t.ratio = -1.33, p = 0.56). These

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interacting effects of the human identity and treatment on behaviour were not found when

508 considering the reunions of the conditioning (𝜒2 < 1.32, p > 0.25 for all CondPCs, table S1).

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509 Interactions between the human identity and conditioning time were not significant, neither

510 considering the reunions of the Isolation/Reunion test (ReuPCs, 2 < 0.642, p > 0.42, tables S1),

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neither the trial number during the session of additional positive contacts of the conditioning

512 (CondPCs, 𝜒2 < 0.11 p > 0.74, table S1).

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1. *Table 5: Significant effects of human identity on vocal response score (VocCP1 and VocPC2) during the reunion of the*
2. *Isolation/Reunion test and during the sessions of additional positive contacts of the conditioning. Only significant effect*
3. *are presented here but a full statistical report is available as supplementary material (tables S1 and S2 for statistical*
4. *tests, table S3 for model estimates). When single effects were interpretable, the Chi-squared statistic are reported. When*
5. *significant interactions were significant, post hoc pairwise comparisons were performed with Tukey corrected and are*
6. *thus reported. The estimates correspond either to the group estimate and comparisons of groups (categorical fixed effect)*
7. *or slope estimates and comparison of slopes (continuous fixed effect, ‘Trial number’).*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Vocal** |  | | | | | | |
| **response** | **Fixed** |  | **Estimat** | **Lower.95%** | **Upper.95%C** |  |  |
| **score** | **effect** | **Levels** | **e** | **CI** | **I** | **Statistic** | **P-value** |

**Reunion of the Isolation/Reunion test**

|  |  |  |  |
| --- | --- | --- | --- |
| AH  VocPC2 humanID | 0.154 | -0.119 |  |
| AV | -0.292 | -0.571 | 1  -0.012 |

**Sessions of additional positive contacts of the conditioning**

0.427 𝜒2 = 4.94 P = 0.03

-VocPC1 humanID AH 0.091 0.037 0.144 𝜒2 = 4.69 P = 0.03

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|  |  |  |  |
| --- | --- | --- | --- |
| AV | 0.076 | 0.021 | 0.132 |
| AH – away  humanID\*I AH – close | 0.317  0.402 | 0.110  0.161 | 0.524  Z-ratio = -1.23 P = 0.60  0.643 |
| VocPC2 nProxArea  AV – away  AV – close | 0.027  0.462 | -0.182  0.212 | 0.236  Z-ratio = -5.77 P < 0.001  0.712 |
| AH – Trial number | -0.048 | -0.070 | -0.026 |
| VocPC3 humanID \*  Trial AV – Trial |  |  |  |
| number number | -0.007 | -0.031 | 0.016 Z-ratio = -2.82 P = 0.005 |

1. 521
2. Considering the vocal scores, no effect of human identity was found on VocPC1 during the
3. Isolation/Reunion tests but -VocPC1 was higher when the human ‘AH’ was in the room during the
4. reunion periods of the conditioning (table 5), meaning the frequency range and the bandwidth of the
5. grunt were higher when the human ‘AH’ interacted with the piglet compared to the human ‘AV’.
6. VocPC2 was higher when the human ‘AH’ was in the room during the Isolation/Reunion tests (table

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5), meaning that the pitch of grunts was higher and this effect was also found during the sessions of

additional positive contacts of the conditioning in interaction with the location of the piglet ( 2 = 11.9, p = 0.001): VocPC2 increased when piglets were located close to the human but this increase was significant only for the human ‘AV’ and not for ‘AH’ (table 5). VocPC3 was not different between humans during the reunions of the Isolation/Reunion tests but, over the conditioning,

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VocPC3 changed differently when piglets were handled by the human ‘AH’ or ‘AV’, as showed by

1. the significant interaction between trial number and human identity (𝜒2 = 8.0, p = 0.005): the

1

1. skewness and kurtosis of grunts decreased over the conditioning when ‘AH’ was interacting with the
2. piglets, but not ‘AV’ (see slope estimates, table 5). No evidence of any effect of human identity was
3. found on grunt duration neither during the Isolation/Reunion tests nor during the sessions of
4. additional positive contacts of the conditioning (table S1).

# Discussion

### Behavioural evidence of a rapid establishment of interest and proximity toward a human

1. ***providing additional positive contacts***
2. The standard reunion test with the human before the conditioning showed that the treatment of
3. positive handling at weaning succeeded in creating two different levels of human-piglet relationship
4. (H and H+), as positively handled piglets expressed a higher attraction toward the human than non
5. handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al.,
6. 2020). This test also showed that the conditioning modified the behaviour of non handled piglets so
7. that they finally expressed a similar attraction toward the human as positively handled piglets, after
8. the conditioning. These results are in line with the behavioural results of the sessions of additional
9. positive contacts. The analysis of piglets’ behaviour every second sessions of the conditioning
10. showed that, although positively handled and non handled piglets started with different degree of
11. proximity toward the human (trials 2 and 4, CondPC1), then, over time and for both treatments (H
12. and H+), piglets expressed a higher attraction toward the human (CondPC1) and avoided less the
13. human when the latter attempted to interact with them. At the end of the conditioning, piglets from
14. both groups had similar level of proximity toward the human (trials 8, 10, 11 CondPC1). So it seems
15. that the conditioning process allowed non handled piglets to compensate the lack of positive
16. handling before the conditioning and develop a similar proximity toward the human. Two minute
17. daily sessions of additional positive contacts changed positively the perception of the human for the
18. piglets, and thus their willingness to interact with them. Since no evidence of any interaction
19. between time and treatment was found, no conclusion on differential developmental trajectories
20. between treatments can be drawn, but a parallel development of the human-piglet relationship in
21. both groups, when considering the proximity.
22. Beside behavioural proximity, piglets that were positively handled at weaning expressed more
23. exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also
24. observed during the sessions of additional positive contacts of the conditioning: positive handled
25. piglets started with a higher score associated with investigation than non handled piglets (CondPC2)
26. and it held over the conditioning. Piglets that were positively handled at weaning also expressed a
27. higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an
28. expression of natural foraging and disinterest from human contact, which may be a sign of positive
29. welfare (Weerd & Day, 2009). In addition, this could also be interpreted in terms of attachment to
30. the human. Indeed, attachment to a human may facilitate exploration of novel environments or
31. objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may
32. provide an environment secure enough for the piglets to explore their environment in the presence of
33. the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al.,

573 2009).

1. Overall, the behavioural monitoring showed that two minute sessions of positive additional contacts
2. per day are sufficient to increase proximity to a human to similar levels as when piglets were
3. previously familiarised for 2 weeks, even when piglets experienced social isolation. But it did not
4. allow the non handled piglets to express natural exploratory behaviours as the positively handled
5. piglets. We may be able to hypothesize a sequential establishment of a positive HAR over time:
6. firstly with a decrease of attentive state and an increase in proximity and accepted contacts, and
7. secondly with a disinterest of human contacts and the expression of natural foraging behaviour. The
8. latter may require a higher exposure time.

### Links between vocal expression and positive HAR

1. In this study, piglets were subjected to two types of interactions with the human: one in a standard
2. reunion test during which, no movements nor speech was produced by the human. The second type
3. of reunion consisted of sessions of additional positive contacts, during which the human actively
4. interacted with the piglet, providing contacts and producing speech. These two types of interactions
5. had different effects on vocal expression, which allows us to evaluate the origin and functions of the
6. vocal flexibility expressed in grunt structure. In the next two paragraphs, we discuss the results of the
7. standard reunion test before and after conditioning. This enables us to raise two possible hypotheses.
8. We then use the results of the sessions of additional positive contacts of the conditioning to discuss
9. theses hypotheses.
10. Human mere presence affects vocal expression according to previous experience
11. Social isolation was associated with longer and lower pitched grunts with a downshifted frequency
12. spectrum. A reunion with a static human changed grunt structure to shorter, higher pitched with an
13. upshifted frequency spectrum and this was observed independently from the treatment (H or H+) and
14. the conditioning time (before or after). In terms of emotional indicators, similar changes in acoustic
15. features of grunts were already found in studies focusing on vocal markers of valence in pigs
16. (Briefer et al., 2019, 2022; Friel et al., 2019; Villain et al., 2020) and are also in line with previous
17. results in similar contexts in relation to the human presence (Villain et al., 2020b). These results, 600 combined with the behavioural results, may validate The symmetrical distribution of some vocal 601 parameters encoding of positive emotions (shorter and higher pitched grunts) in the context of a 602 reunion with a human, potentially releasing the piglets from the stress of isolation.

603 In addition, and surprisingly, positively handled piglets produced shorter grunts than non handled 604 piglets whatever the context of observation (with or without human presence). This was previously 605 shown in another context (anticipation of (pseudo)social events independently from the type of 606 partner) in the same groups of piglets (Villain et al. 2020). This may show that the period of positive 607 handling at weaning modulated general vocal production in the studied pigs in the long term. To our 608 knowledge, long-term effects of a period of positive handling at weaning on grunt duration has not 609 been studied to confront this finding to the literature.

610 We showed that the proximity to the human changed the structure of piglets grunts and that 611 proximity significantly interacted with the treatment and the conditioning time. Indeed, similarly to a 612 previous study (Villain et al. 2020a, 2020b), during the standard reunion test (no contact from the 613 human), piglets produced shorter and higher pitched grunts with an upshifted frequency spectrum 614 when closer to the human. It was especially the case in non handled piglets, before the conditioning. 615 These results may be linked to the reactivity to the human, non handled piglets being more reactive 616 to the presence of a human than positively handled piglets. Indeed, before the sessions of the 617 conditioning, positively handled piglets were habituated to a human interacting positively when 618 present whereas non handled piglets were not, hence, during the first standard reunion test, when the 619 human is present but do not interact with the piglet, positively handled and non handled piglets may 620 have diverging expectations regarding the presence of the static and silent human. As positively 621 handled piglets received positive contacts every time they were in the presence of the human, they

622 may have expected positive contacts when approaching and experienced an absence of reward during 623 the test. This has already been hypothesised in piglets deprived from human voice during interactions 624 after a period of habituation to it (Bensoussan et al. 2020). On the contrary, piglets that were not 625 positively handled at weaning never experienced additional positive contacts and being close to a 626 human, having the possibility to investigate them may be some kind of reward after the period of 627 total isolation. After the conditioning, piglets from both treatments were conditioned to receive 628 additional positive contacts and both groups had experienced a first standard reunion test, so they 629 may both experience an absence of reward during the test, which may explain a lower reaction to 630 proximity to the human, and thus fewer changes on grunt spectro-temporal features.

631 The results on vocal expression during the standard reunion test show that even in a context in which 632 the human does not provide positive contacts, the early experience the piglets had with them affected 633 their vocal expression when close to them and on the long-term. On the one hand, we know that a 634 positive HAR establishes through successive positive experiences (Rault et al. 2020) and, on the 635 other hand, HAR may have long term effects on behavioural expressions, as suggested by Brajon et 636 al. (2015) using cognitive bias tests. We can thus hypothesize this may also be reflected in the way 637 piglets vocalise. In that case, we may have evidence of expression of another category of affect, 638 moods, and not only emotional expression. Indeed, as suggested by Schnall (2010), although 639 emotions are short-term affects triggered by an external stimulus, moods, on the other hand, may be 640 experienced on a longer term and may not be attributable to a specific stimulus. Although emotions 641 and moods do not rely on the same time scale, they may interact with one another, and more studies 642 are needed to understand their effects on vocal expression.

643 This test may allow us to suggest two potential non-exclusive hypotheses to explain why the effect of 644 human proximity on grunt acoustic structure attenuates as the familiarity to the human increases. In a 645 first hypothesis, we could think that this attenuation of acoustic flexibility is due to a decrease in 646 reactivity to the human, which may be linked to a disinterest of human contacts and an increase in 647 natural foraging behaviours. In another hypothesis, this attenuation of acoustic flexibility may be due 648 to a violation of piglets expectations: because the human remains static during the test, this may 649 inhibit vocal reactions to the proximity. The interpretation of the second type of human-piglet 650 interactions below may allow us to address these hypotheses.

651 Providing rewarding additional positive contacts changes the structure of grunts

652 During the sessions of additional positive contacts of the conditioning, independently from the trial

653 number, the duration of grunts was lower when the piglets were located close to the human and

654 similarly short in both experimental treatments, but when away from the human, non handled piglets 655 had longer grunts than positively handled piglets. In addition, although the pitch (VocPC2) and the 656 frequency distribution (-VocPC1) increased over the conditioning, it remained higher when grunts 657 were produced closer to the human and especially in non handled piglets (VocPC2). These results are 658 in line with the behavioural results showing an increase in proximity to the human over the 659 conditioning (CondPC1). During this type of interaction, and contrary to the standard reunion test, 660 changes in acoustic features of grunts when close to the human were consistent over the 661 conditioning: piglets remained reactive to the proximity to the human over time. This result may not 662 be in line with the first hypothesis: in the case of a human interacting with a piglet, the effect of the 663 proximity does not seem to attenuate over time. Hence, the behaviour of the human during a session 664 impacts the way a piglet vocalises. In that case, we may raise two more hypotheses to explain this 665 vocal behaviour : either it is linked to the emotional state or it is linked to a specific human-animal 666 communication, the two explanations may not be exclusive. The first possible explanation may be 667 linked to the emotional state experienced by the piglets when approaching a human providing 668 additional positive contact. As a reminder, in the context of the session, the piglet can choose to 669 approach and stay close to the human, which will provide positive contacts systematically. So the 670 piglet may anticipate to receive positive contact and systematically being rewarded. When close to 671 the human, observed changes in frequency distribution of grunts (increased pitch and upshifted 672 frequency spectrum) are known to be markers of arousal (in multiple mamalian species (Briefer, 673 2012, 2020) and pigs (Linhart et al., 2015)). As a consequence, this may show that pigs enter a state 674 of higher arousal when being close to a carrying human. To go further, we show that these spectral 675 changes were also associated with shorter grunts. Although the duration of grunts is associated with 676 the valence of a situation, it is not clear whether the duration could also be an indicator of the 677 arousal; if our hypothesis is true then it would be the first demonstration that shorter grunts are also 678 indicators of higher arousal positive state in pigs. Last, we can raise the question whether changes in 679 grunt structure may also be associated with a specific human-pig communication. In other domestic 680 species, owner directed vocalisations has been shown (in cats, reviewed in (Turner, 2017); in dogs 681 (Gaunet et al., 2022)). In addition, studies have found similar socio-communicative behaviours 682 toward a human in socialized pigs and dogs (Gerencsér et al., 2019). Hence, we may profit from 683 testing the existence of human directed vocalisations in pigs, as consequences of their socio 684 communicative abilities.

### 685 Effect of human identity on piglets’ perception: perspectives on HAR

686 We found that the identity of the human had effects on behavioural and vocal response scores. 687 Piglets that were handled by the human ‘AH’ had higher values of behavioural proximity (ReuPC1) 688 than piglets handled by the human ‘AV’ during reunion test after a period of isolation. This effect 689 was not found during conditioning sessions. The effect of the human did not interact with the 690 conditioning time, leading to the conclusion that the difference between the two experimenter may 691 have establishment during the period of positive handling at weaning, prior to the conditioning. 692 Additionally, when the human ‘AH’ was in the room, piglets produced grunts with a more upshifted 693 frequency spectrum and a higher pitch than when the human ‘AV’ was in the room. If upshifted 694 grunts may be a indicator of positive higher arousal, then we may conclude that ‘AH’ was more 695 likely to trigger higher positive states than ‘AV’. Interestingly, the human identity and the spatial 696 proximity had different effects on piglets grunts during sessions of additional positive contacts but 697 not when the human was static during the standard reunion test. Hence, it is possible that the way one 698 human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering 699 positive emotions and thus modifications of grunt structure. Several evidence exists in the literature 700 that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al., 701 2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We 702 may question the efficiency of different human features to generate a positive HAR. In our study, 703 both humans that interacted with the piglets wear exactly the same clothes and standardized their 704 tactile interactions toward the piglets before starting the study, and agreed on the rhythm and types of 705 sounds (words, intonation) to use, to minimise generating variability although no systematic controls 706 of the human behaviour or spectral feature of voices were performed here. It thus remains unclear 707 whether experimenters interacted differently or if they were initially perceived differently by piglets. 708 Our results show that the identity of the human may modulate piglet proximity and vocal behaviour 709 but the design of this experiment does not allow to find the causes of these observations (behaviour, 710 voice characteristics, or even odour profile). Thus, more studies of human features that are most 711 likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In 712 addition, studying human-piglet relationship in a more systematic way, as in other domestic species, 713 for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech 714 (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies 715 of interspecific relationships. However, the influence of human identity did not modify the general 716 outcomes of our study, but only decreased some effects, suggesting that this variability does not 717 modify the main results, but should be considered in future studies.

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719 To conclude, we showed that degrees of familiarity toward a human could be reflected in the way 720 piglets vocalise in their presence, and out of it. We also showed that the spatial proximity toward a 721 human providing additional care could change the acoustic structure of piglet grunts. These changes 722 are likely to be linked to positive and more intense emotional states than when piglets are further 723 away from the human. However, it is still unclear whether the changes in grunt structure could also 724 be linked to human-animal communication and more studies are needed to determine it. We did also 725 show that the identity of the human may be of importance, and may generate vocal changes during 726 additional positive contacts that were not associated with changes in behaviour of the human. More 727 systematic studies of human behaviour along with pig behaviour during the human-animal 728 interactions would be needed to have a better understanding of the evolution of HAR, especially 729 interactive interspecific communication as well as providing new procedures to promote positive 730 welfare. We suggest that analysing vocalisations structure may be a good tool to assess the quality of 731 human-pig relationship and help monitor the establishment of a positive HAR.

# 732 Authors contributions

733 Conceived and designed the experiment (A.V., C.T., C.N.). Performed the experiment (A.V., C.G.). 734 Collection and processing of the acoustic and behavioural data (A.V., C.G.). Statistical analyses 735 (A.V.). Contributed to the writing of the manuscript (A.V., C.T., C.N.).

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# 744 Data availability

745 The datasets used for the study are available at (Villain et al., 2022). The folder contains all datasets 746 and a readme to match the type of analysis to the proper dataset. We have made sure to report in the 747 main text of the article which R libraries and which functions in these libraries we used. All formulas

748 of the statistical models are explicit in the text to facilitate transfer of information and replicate the 749 analysis. All libraries are open source as well.

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**Electronic supplementary material**

Table S1 : Anova table of all models computed. The function ‘Anova’ from the ‘car’ R package was used to generate p value on full models. Interpretable significant p-values are bolded. When significant interaction were found, post hoc tests were performed (see table S2).

|  |  |  |  |
| --- | --- | --- | --- |
| **Fixed effects** | **Chisq** | **Df** | **Pr..Chisq.** |
| **Model #1 : behavioural response Reunion of the Isolation/Reunion test** | | | |
| **ReuPC1**  Treatment | 19.077 | 1 | <0.001 |
| Conditioning time | 139.035 | 1 | <0.001 |
| Batch | 42.566 | 1 | <0.001 |
| HumanID | 0.137 | 1 | 0.711 |
| Treatment:Conditioning time | 27.910 | 1 | **<0.001** |
| Treatment:Batch | 0.507 | 1 | 0.476 |
| Treatment:HumanID | 6.009 | 1 | **0.014** |
| Conditioning time:Batch | 20.240 | 1 | **<0.001** |
| Conditioning time:HumanID | 0.646 | 1 | 0.422 |
| **ReuPC2 (sqrt+4)** |  |  |  |
| Treatment | 0.995 | 1 | 0.319 |
| Conditioning time | 3.782 | 1 | 0.052 |
| Batch | 5.118 | 1 | 0.024 |
| HumanID | 1.978 | 1 | 0.160 |
| Treatment:Conditioning time | 0.000 | 1 | 0.989 |
| Treatment:Batch | 0.333 | 1 | 0.564 |
| Treatment:HumanID | 1.752 | 1 | 0.186 |
| Conditioning time:Batch | 14.193 | 1 | **<0.001** |
| Conditioning time:HumanID | 0.189 | 1 | 0.663 |
| **-ReuPC3 (sqrt +3)** |  |  |  |
| Treatment | 6.884 | 1 | 0.009 |
| Conditioning time | 31.456 | 1 | <0.001 |
| Batch | 0.000 | 1 | 0.984 |
| HumanID | 0.385 | 1 | 0.535 |
| Treatment:Conditioning time | 3.658 | 1 | 0.056 |
| Treatment:Batch | 6.966 | 1 | **0.008** |
| Treatment:HumanID | 2.010 | 1 | 0.156 |
| Conditioning time:Batch | 5.445 | 1 | **0.020** |
| Conditioning time:HumanID | 0.247 | 1 | 0.619 |

**Model #2 : Vocal response Isolation/Reunion tests : Treatment \* Phase \* Conditioning time**

|  |  |  |  |
| --- | --- | --- | --- |
| **Call duration (s) (log)** |  | | |
| Treatment | 5.503 | 1 | **0.019** |
| Phase | 60.842 | 1 | **<0.001** |
| Conditioning time | 62.883 | 1 | <0.001 |
| HumanID | 0.535 | 1 | 0.465 |

|  |  |  |  |
| --- | --- | --- | --- |
| Batch | 8.053 | 1 | 0.005 |
| Treatment:Phase | 0.872 | 1 | 0.350 |
| Treatment:Conditioning time | 3.479 | 1 | 0.062 |
| Phase:Conditioning time | 1.894 | 1 | 0.169 |
| Treatment:HumanID | 0.048 | 1 | 0.826 |
| Conditioning time:HumanID | 2.347 | 1 | 0.126 |
| Treatment:Batch | 2.398 | 1 | 0.121 |
| Conditioning time:Batch | 10.844 | 1 | **0.001** |
| Treatment:Phase:Conditioning time | 0.699 | 1 | 0.403 |
| **-VocPC1** |  |  |  |
| Treatment | 0.886 | 1 | 0.346 |
| Phase | 8.501 | 1 | **0.004** |
| Conditioning time | 0.359 | 1 | 0.549 |
| HumanID | 2.519 | 1 | 0.112 |
| Batch | 60.781 | 1 | **<0.001** |
| Treatment:Phase | 0.735 | 1 | 0.391 |
| Treatment:Conditioning time | 0.592 | 1 | 0.442 |
| Phase:Conditioning time | 0.616 | 1 | 0.433 |
| Treatment:HumanID | 0.095 | 1 | 0.758 |
| Conditioning time:HumanID | 0.786 | 1 | 0.375 |
| Treatment:Batch | 0.129 | 1 | 0.720 |
| Conditioning time:Batch | 1.875 | 1 | 0.171 |
| Treatment:Phase:Conditioning time | 0.000 | 1 | 0.995 |
| **VocPC2** |  |  |  |
| Treatment | 0.011 | 1 | 0.918 |
| Phase | 19.116 | 1 | **<0.001** |
| Conditioning time | 245.911 | 1 | <0.001 |
| HumanID | 6.152 | 1 | **0.013** |
| Batch | 2.378 | 1 | 0.123 |
| Treatment:Phase | 3.525 | 1 | 0.060 |
| Treatment:Conditioning time | 0.695 | 1 | 0.405 |
| Phase:Conditioning time | 2.105 | 1 | 0.147 |
| Treatment:HumanID | 0.280 | 1 | 0.597 |
| Conditioning time:HumanID | 0.032 | 1 | 0.858 |
| Treatment:Batch | 0.355 | 1 | 0.552 |
| Conditioning time:Batch | 34.561 | 1 | **<0.001** |
| Treatment:Phase:Conditioning time | 0.314 | 1 | 0.576 |
| **-VocPC3** |  |  |  |
| Treatment | 4.782 | 1 | **0.029** |
| Phase | 6.567 | 1 | **0.010** |
| Conditioning time | 2.945 | 1 | 0.086 |
| HumanID | 0.870 | 1 | 0.351 |
| Batch | 50.730 | 1 | <0.001 |
| Treatment:Phase | 0.721 | 1 | 0.396 |
| Treatment:Conditioning time | 0.102 | 1 | 0.750 |
| Phase:Conditioning time | 2.026 | 1 | 0.155 |
| Treatment:HumanID | 0.087 | 1 | 0.767 |
| Conditioning time:HumanID | 2.002 | 1 | 0.157 |
| Treatment:Batch | 1.905 | 1 | 0.168 |

|  |  |  |  |
| --- | --- | --- | --- |
| Conditioning time:Batch | 8.468 | 1 | **0.004** |
| Treatment:Phase:Conditioning time | 0.624 | 1 | 0.429 |

**Model #3 : Vocal response Reunion of the Isolation/Reunion tests : conditioning time \* Treatment \* In prox. area**

|  |  |  |  |
| --- | --- | --- | --- |
| **Call duration (s) (log)**  Conditioning time | 37.742 | 1 | <0.001 |
| Treatment | 0.943 | 1 | 0.331 |
| In prox. area | 48.590 | 1 | <0.001 |
| HumanID | 2.208 | 1 | 0.137 |
| Batch | 4.987 | 1 | 0.026 |
| Conditioning time:Treatment | 1.892 | 1 | 0.169 |
| Conditioning time:In prox. area | 4.913 | 1 | 0.027 |
| Treatment:In prox. area | 16.021 | 1 | <0.001 |
| Conditioning time:HumanID | 0.526 | 1 | 0.468 |
| Conditioning time:Batch | 29.430 | 1 | **<0.001** |
| Treatment:Batch | 0.172 | 1 | 0.678 |
| Treatment:HumanID | 0.004 | 1 | 0.947 |
| In prox. area:HumanID | 2.058 | 1 | 0.151 |
| In prox. area:Batch | 0.010 | 1 | 0.919 |
| Conditioning time:Treatment:In prox. area | 6.541 | 1 | **0.011** |
| **-VocPC1** |  |  |  |
| Conditioning time | 0.391 | 1 | 0.532 |
| Treatment | 0.026 | 1 | 0.873 |
| In prox. area | 0.973 | 1 | 0.324 |
| HumanID | 3.006 | 1 | 0.083 |
| Batch | 36.673 | 1 | **<0.001** |
| Conditioning time:Treatment | 0.802 | 1 | 0.371 |
| Conditioning time:In prox. area | 0.600 | 1 | 0.439 |
| Treatment:In prox. area | 14.375 | 1 | <0.001 |
| Conditioning time:HumanID | 0.261 | 1 | 0.609 |
| Conditioning time:Batch | 3.911 | 1 | 0.048 |
| Treatment:Batch | 1.671 | 1 | 0.196 |
| Treatment:HumanID | 0.272 | 1 | 0.602 |
| In prox. area:HumanID | 2.024 | 1 | 0.155 |
| In prox. area:Batch | 2.939 | 1 | 0.086 |
| Conditioning time:Treatment:In prox. area | 4.987 | 1 | **0.026** |
| **VocPC2** |  |  |  |
| Conditioning time | 110.726 | 1 | <0.001 |
| Treatment | 0.351 | 1 | 0.554 |
| In prox. area | 26.883 | 1 | <0.001 |
| HumanID | 4.938 | 1 | **0.026** |
| Batch | 2.275 | 1 | 0.131 |
| Conditioning time:Treatment | 0.016 | 1 | 0.900 |
| Conditioning time:In prox. area | 10.339 | 1 | **0.001** |
| Treatment:In prox. area | 4.225 | 1 | **0.040** |
| Conditioning time:HumanID | 0.037 | 1 | 0.848 |
| Conditioning time:Batch | 37.624 | 1 | **<0.001** |
| Treatment:Batch | 0.342 | 1 | 0.559 |

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment:HumanID | 0.403 | 1 | 0.525 |
| In prox. area:HumanID | 0.020 | 1 | 0.887 |
| In prox. area:Batch | 8.818 | 1 | **0.003** |
| Conditioning time:Treatment:In prox. area | 3.353 | 1 | 0.067 |
| **-VocPC3** |  |  |  |
| Conditioning time | 6.221 | 1 | 0.013 |
| Treatment | 3.158 | 1 | 0.076 |
| In prox. area | 8.537 | 1 | 0.003 |
| HumanID | 1.180 | 1 | 0.277 |
| Batch | 40.179 | 1 | <0.001 |
| Conditioning time:Treatment | 0.371 | 1 | 0.542 |
| Conditioning time:In prox. area | 3.245 | 1 | 0.072 |
| Treatment:In prox. area | 1.308 | 1 | 0.253 |
| Conditioning time:HumanID | 0.154 | 1 | 0.695 |
| Conditioning time:Batch | 8.632 | 1 | **0.003** |
| Treatment:Batch | 2.241 | 1 | 0.134 |
| Treatment:HumanID | 0.046 | 1 | 0.830 |
| In prox. area:HumanID | 2.982 | 1 | 0.084 |
| In prox. area:Batch | 10.363 | 1 | **0.001** |
| Conditioning time:Treatment:In prox. area | 4.893 | 1 | **0.027** |
| **Model #4 : Behavioural response during sessions of conditioning** |  |  |  |
| **CondPC1**  Trial number | 59.317 | 1 | **<0.001** |
| Treatment | 0.128 | 1 | 0.721 |
| HumanID | 1.320 | 1 | 0.251 |
| Batch | 14.497 | 1 | **<0.001** |
| Trial number:Treatment | 2.545 | 1 | 0.111 |
| Trial number:HumanID | 0.023 | 1 | 0.880 |
| Trial number:Batch | 0.626 | 1 | 0.429 |
| Treatment:Batch | 1.663 | 1 | 0.197 |
| Treatment:HumanID | 0.437 | 1 | 0.508 |
| **CondPC2** |  |  |  |
| Trial number | 48.618 | 1 | **<0.001** |
| Treatment | 12.806 | 1 | **<0.001** |
| HumanID | 0.226 | 1 | 0.635 |
| Batch | 10.056 | 1 | **0.002** |
| Trial number:Treatment | 0.041 | 1 | 0.839 |
| Trial number:HumanID | 0.000 | 1 | 0.999 |
| Trial number:Batch | 0.085 | 1 | 0.771 |
| Treatment:Batch | 2.007 | 1 | 0.157 |
| Treatment:HumanID | 0.907 | 1 | 0.341 |
| **CondPC3** |  |  |  |
| Trial number | 0.006 | 1 | 0.939 |
| Treatment | 6.969 | 1 | **0.008** |
| HumanID | 0.375 | 1 | 0.541 |
| Batch | 0.015 | 1 | 0.903 |
| Trial number:Treatment | 0.616 | 1 | 0.432 |
| Trial number:HumanID | 0.109 | 1 | 0.741 |

|  |  |  |  |
| --- | --- | --- | --- |
| Trial number:Batch | 0.166 | 1 | 0.684 |
| Treatment:Batch | 0.078 | 1 | 0.780 |
| Treatment:HumanID | 0.143 | 1 | 0.705 |
| **Missed contact attempts by Human ID (binomial)** |  |  |  |
| Trial number | 23.159 | 1 | <0.001 |
| Treatment | 9.563 | 1 | **0.002** |
| HumanID | 0.463 | 1 | 0.496 |
| Batch | 12.833 | 1 | <0.001 |
| Trial number:Treatment | 0.218 | 1 | 0.640 |
| Trial number:HumanID | 0.058 | 1 | 0.809 |
| Trial number:Batch | 4.485 | 1 | **0.034** |
| Treatment:Batch | 1.274 | 1 | 0.259 |
| Treatment:HumanID | 1.073 | 1 | 0.300 |

**Model #5 : Vocal response during session of conditioning : Trial number \* Treatment \* In prox. area**

|  |  |  |  |
| --- | --- | --- | --- |
| **Call duration (s) (log)**  Trial number | 48.880 | 1 | <0.001 |
| Treatment | 5.192 | 1 | 0.023 |
| In prox. area | 160.565 | 1 | <0.001 |
| HumanID | 0.090 | 1 | 0.765 |
| Batch | 11.814 | 1 | 0.001 |
| Trial number:Treatment | 0.384 | 1 | 0.536 |
| Trial number:In prox. area | 0.584 | 1 | 0.445 |
| Treatment:In prox. area | 15.779 | 1 | **<0.001** |
| Trial number:HumanID | 1.252 | 1 | 0.263 |
| Trial number:Batch | 5.374 | 1 | **0.020** |
| Treatment:Batch | 6.716 | 1 | **0.010** |
| Treatment:HumanID | 0.712 | 1 | 0.399 |
| In prox. area:HumanID | 0.004 | 1 | 0.951 |
| In prox. area:Batch | 0.105 | 1 | 0.746 |
| Trial number:Treatment:In prox. area | 0.019 | 1 | 0.889 |
| **-VocPC1** |  |  |  |
| Trial number | 12.233 | 1 | <0.001 |
| Treatment | 0.043 | 1 | 0.835 |
| In prox. area | 2.225 | 1 | 0.136 |
| HumanID | 4.696 | 1 | **0.030** |
| Batch | 62.339 | 1 | <0.001 |
| Trial number:Treatment | 1.091 | 1 | 0.296 |
| Trial number:In prox. area | 3.968 | 1 | **0.046** |
| Treatment:In prox. area | 1.089 | 1 | 0.297 |
| Trial number:HumanID | 0.155 | 1 | 0.694 |
| Trial number:Batch | 0.099 | 1 | 0.753 |
| Treatment:Batch | 6.990 | 1 | **0.008** |
| Treatment:HumanID | 0.606 | 1 | 0.436 |
| In prox. area:HumanID | 2.204 | 1 | 0.138 |
| In prox. area:Batch | 5.703 | 1 | **0.017** |
| Trial number:Treatment:In prox. area | 0.275 | 1 | 0.600 |
| **VocPC2** |  |  |  |
| Trial number | 85.956 | 1 | <0.001 |

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | 0.217 | 1 | 0.642 |
| In prox. area | 13.440 | 1 | <0.001 |
| HumanID | 2.932 | 1 | 0.087 |
| Batch | 6.712 | 1 | 0.010 |
| Trial number:Treatment | 0.507 | 1 | 0.477 |
| Trial number:In prox. area | 6.107 | 1 | **0.013** |
| Treatment:In prox. area | 7.622 | 1 | **0.006** |
| Trial number:HumanID | 0.016 | 1 | 0.899 |
| Trial number:Batch | 8.261 | 1 | **0.004** |
| Treatment:Batch | 1.482 | 1 | 0.223 |
| Treatment:HumanID | 2.318 | 1 | 0.128 |
| In prox. area:HumanID | 11.895 | 1 | **0.001** |
| In prox. area:Batch | 1.792 | 1 | 0.181 |
| Trial number:Treatment:In prox. area | 0.049 | 1 | 0.824 |
| **VocPC3** |  |  |  |
| Trial number | 14.564 | 1 | <0.001 |
| Treatment | 2.710 | 1 | 0.100 |
| In prox. area | 4.578 | 1 | 0.032 |
| HumanID | 0.652 | 1 | 0.419 |
| Batch | 44.701 | 1 | **<0.001** |
| Trial number:Treatment | 2.485 | 1 | 0.115 |
| Trial number:In prox. area | 0.315 | 1 | 0.575 |
| Treatment:In prox. area | 2.502 | 1 | 0.114 |
| Trial number:HumanID | 7.978 | 1 | **0.005** |
| Trial number:Batch | 0.456 | 1 | 0.499 |
| Treatment:Batch | 0.029 | 1 | 0.865 |
| Treatment:HumanID | 0.000 | 1 | 0.984 |
| In prox. area:HumanID | 1.696 | 1 | 0.193 |
| In prox. area:Batch | 0.001 | 1 | 0.970 |
| Trial number:Treatment:In prox. area | 0.183 | 1 | 0.669 |

Table S2 : Table of contrasts from posthoc tests following significant interaction using the Anova on the model, pairwise comparison with Tukey correction. P-values were generated using the ‘emmeans’ (categorical fixed effect) and ‘lstrends’ (continuous fixed effect) functions of the ‘Emmeans’ R package. Estimates indicated are either between groups or slope comparisons, depending on the categorial or continuous variable (trial number). When fixed effect interacting with the batch, the batch number was fixed to compare the fixed effect within each batch. When three way interaction were significant, one factor was fixed to compare the interacting effect of the two other (conditioning time was fixed).

**contrast**

**fixed comparison**

**factor if any estimate SE ratio p.value**

**Model #1 : behavioural response of the Reunion of Isolation/Reunion test**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **ReuPC1**  Treatment \* Conditioning time |  | | | | |
| H after - H+ after | - | 0.022 | 0.311 | 0.072 | 1.000 |
| H after - H before | - | 3.430 | 0.284 | 12.073 | <0.001 |
| H after - H+ before | - | 1.330 | 0.311 | 4.271 | <0.001 |
| H+ after - H before | - | 3.407 | 0.311 | 10.945 | <0.001 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| H+ after - H+ before |  | 1.307 | 0.284 | 4.602 |  |
| H before – H+ before | - | -2.100 | 0.311 | -6.746 | <0.001 |
| Conditioning time | Batch  after - before | 1 | 1.446 | 0.287 | 5.039 | <0.001 |
| after - before  Treatment \* HumanID | 2 | 3.291 | 0.287 | 11.466 | <0.001 |
| H AH - H+ AH |  | -1.634 | 0.340 | -4.808 | <0.001 |
| H AH - H AV |  | -0.685 | 0.343 | -1.995 | 0.202 |
| H AH - H+ AV |  | -1.129 | 0.340 | -3.321 | 0.009 |
| H+ AH - H AV |  | 0.949 | 0.340 | 2.792 | 0.035 |
| H+ AH - H+ AV |  | 0.505 | 0.343 | 1.471 | 0.462 |
| H AV - H+ AV |  | -0.444 | 0.340 | -1.306 | 0.563 |
| **ReuPC2 (sqrt+4)** |  |  |  |  |  |
| Conditioning time | Batch after - before | 1 | 0.137 | 0.103 | 1.330 | 0.189 |
| after - before | 2 | -0.416 | 0.103 | -4.052 | <0.001 |
| **-ReuPC3 (sqrt +3)** |  |  |  |  |  |
| Treatment \* Conditioning time H after - H+ after | - | -0.252 | 0.078 | -3.237 | 0.009 |
| H after - H before | - | 0.187 | 0.072 | 2.613 | 0.054 |
| H after - H+ before | - | 0.129 | 0.078 | 1.657 | 0.352 |
| H+ after - H before | - | 0.439 | 0.078 | 5.642 | <0.001 |
| H+ after - H+ before | - | 0.381 | 0.072 | 5.318 | <0.001 |
| H before - H+ before | - | -0.058 | 0.078 | -0.748 | 0.877 |
| Treatment | Batch  H - H+ | 1 | -0.314 | 0.084 | -3.721 | <0.001 |
| H - H+ | 2 | 0.004 | 0.084 | 0.049 | 0.961 |
| Conditioning time | Batch  after - before | 1 | 0.404 | 0.072 | 5.592 | <0.001 |
| after - before | 2 | 0.163 | 0.072 | 2.258 | 0.028 |

**Model #2 : Vocal response Isolation/Reunion tests : Treatment \* Phase \* Conditioning time**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Call duration (s) (log)**  Conditioning time | Batch |  | | | | |
| after - before | 1 | 0.171 | 0.045 | 3.760 | <0.001 |
| after - before | 2 | 0.398 | 0.052 | 7.680 | <0.001 |
| **VocPC2** |  |  |  |  |  |
| Conditioning time | Batch after - before | 1 | -0.832 | 0.101 | -8.232 | <0.001 |
| after - before | 2 | -1.755 | 0.120 | -14.595 | <0.001 |
| **-VocPC3** |  |  |  |  |  |
| Conditioning time | Batch after - before | 1 | 0.308 | 0.100 | 3.085 | 0.002 |
| after - before | 2 | -0.136 | 0.115 | -1.181 | 0.238 |

**Model #3 : Vocal response during the Reunion of Isolation/Reunion tests : conditioning time \* Treatment \* In prox. area**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Call duration (s) (log)**  Treatment \* In prox. area | conditioning time |  |  |  |  |  |
| H 0 - H+ 0 | after | 0.040 | 0.071 | 0.564 | 0.943 |
| H 0 - H 1 | after | 0.123 | 0.030 | 4.097 | <0.001 |
| H 0 - H+ 1 | after | 0.090 | 0.073 | 1.224 | 0.612 |
| H+ 0 - H 1 | after | 0.083 | 0.074 | 1.115 | 0.680 |
| H+ 0 - H+ 1 | after | 0.050 | 0.025 | 1.989 | 0.192 |
| H 1 - H+ 1 | after | -0.033 | 0.076 | -0.433 | 0.973 |
| H 0 - H+ 0 | before | 0.187 | 0.079 | 2.384 | 0.080 |
| H 0 - H 1 | before | 0.312 | 0.049 | 6.329 | <0.001 |
| H 0 - H+ 1 | before | 0.254 | 0.080 | 3.185 | 0.008 |
| H+ 0 - H 1 | before | 0.124 | 0.088 | 1.418 | 0.488 |
| H+ 0 - H+ 1 | before | 0.066 | 0.030 | 2.186 | 0.127 |
| H 1 - H+ 1 | before | -0.058 | 0.087 | -0.664 | 0.911 |
| Conditioning time | Batch |  |  |  |  |  |
| after - before | 1 | 0.084 | 0.049 | 1.734 | 0.083 |
| after - before | 2 | 0.492 | 0.059 | 8.334 | <0.001 |
| **-VocPC1** |  |  |  |  |  |
| Treatment \* In prox. area | conditioning time |  |  |  |  |  |
| H 0 - H+ 0 | after | -0.345 | 0.416 | -0.829 | 0.841 |
| H 0 - H 1 | after | -0.227 | 0.184 | -1.232 | 0.607 |
| H 0 - H+ 1 | after | -0.124 | 0.431 | -0.288 | 0.992 |
| H+ 0 - H 1 | after | 0.118 | 0.436 | 0.271 | 0.993 |
| H+ 0 - H+ 1 | after | 0.221 | 0.153 | 1.443 | 0.473 |
| H 1 - H+ 1 | after | 0.103 | 0.450 | 0.228 | 0.996 |
| H 0 - H+ 0 | before | -0.052 | 0.473 | -0.109 | 1.000 |
| H 0 - H 1 | before | -1.014 | 0.304 | -3.340 | 0.005 |
| H 0 - H+ 1 | before | 0.311 | 0.480 | 0.647 | 0.917 |
| H+ 0 - H 1 | before | -0.962 | 0.531 | -1.811 | 0.268 |
| H+ 0 - H+ 1 | before | 0.362 | 0.187 | 1.939 | 0.212 |
| H 1 - H+ 1 | before | 1.324 | 0.530 | 2.500 | 0.060 |
| Conditioning time | Batch |  |  |  |  |  |
| after - before | 1 | 0.552 | 0.395 | 1.397 | 0.162 |
| after - before | 2 | -0.635 | 0.458 | -1.387 | 0.166 |

**VocPC2\***

\*Note : due to a three way interaction close to significance level, contrasts were generating with the three way interaction or with the two ways interactions of interests

Treatment \* In prox. area | conditioning time

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| H 0 - H+ 0 | after | 0.112 | 0.215 | 0.519 | 0.955 |
| H 0 - H 1 | after | -0.216 | 0.093 | -2.326 | 0.092 |
| H 0 - H+ 1 | after | -0.022 | 0.223 | -0.100 | 1.000 |
| H+ 0 - H 1 | after | -0.328 | 0.225 | -1.454 | 0.466 |
| H+ 0 - H+ 1 | after | -0.134 | 0.077 | -1.733 | 0.306 |
| H 1 - H+ 1 | after | 0.193 | 0.232 | 0.834 | 0.838 |
| H 0 - H+ 0 | before | -0.016 | 0.238 | -0.067 | 1.000 |
| H 0 - H 1 | before | -0.798 | 0.152 | -5.239 | <0.001 |
| H 0 - H+ 1 | before | -0.351 | 0.241 | -1.454 | 0.466 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| H+ 0 - H 1 | before | -0.783 | | 0.267 | -2.933 | 0.018 |
| H+ 0 - H+ 1 | before | -0.335 | | 0.094 | -3.561 | 0.002 |
| H 1 - H+ 1 | before | 0.448 | | 0.266 | 1.685 | 0.332 |
| In prox. area | Conditioning time |  |  | |  |  |  |
| 0 - 1 | after | -0.175 | | 0.061 | -2.876 | 0.004 |
| 0 - 1 | before | -0.567 | | 0.093 | -6.124 | <0.001 |
| Treatment \* In prox. area |  |  | |  |  |  |
| H 0 - H+ 0 |  | 0.048 | | 0.198 | 0.242 | 0.995 |
| H 0 - H 1 |  | -0.507 | | 0.092 | -5.539 | 0.000 |
| H 0 - H+ 1 |  | -0.187 | | 0.202 | -0.926 | 0.791 |
| H+ 0 - H 1 |  | -0.555 | | 0.211 | -2.634 | 0.042 |
| H+ 0 - H+ 1 |  | -0.235 | | 0.061 | -3.827 | 0.001 |
| H 1 - H+ 1 |  | 0.320 | | 0.212 | 1.513 | 0.429 |
| In prox. area | Batch |  |  | |  |  |  |
| 0 - 1 | 1 | -0.223 | | 0.064 | -3.480 | 0.001 |
| 0 - 1 | 2 | -0.519 | | 0.086 | -6.051 | <0.001 |
| Conditioning time | Batch |  |  | |  |  |  |
| after - before | 1 | -0.680 | | 0.140 | -4.864 | <0.001 |
| after - before | 2 | -2.009 | | 0.172 | -11.710 | <0.001 |
| **-VocPC3** |  |  | |  |  |  |
| Treatment \* In prox. area |  |  | |  |  |  |
| H 0 - H+ 0 | after | 0.227 | | 0.132 | 1.726 | 0.310 |
| H 0 - H 1 | after | 0.125 | | 0.073 | 1.703 | 0.322 |
| H 0 - H+ 1 | after | 0.324 | | 0.139 | 2.332 | 0.091 |
| H+ 0 - H 1 | after | -0.102 | | 0.142 | -0.722 | 0.888 |
| H+ 0 - H+ 1 | after | 0.097 | | 0.061 | 1.580 | 0.390 |
| H 1 - H+ 1 | after | 0.199 | | 0.148 | 1.345 | 0.534 |
| H 0 - H+ 0 | before | 0.033 | | 0.153 | 0.218 | 0.996 |
| H 0 - H 1 | before | -0.266 | | 0.120 | -2.207 | 0.121 |
| H 0 - H+ 1 | before | 0.105 | | 0.156 | 0.674 | 0.907 |
| H+ 0 - H 1 | before | -0.299 | | 0.179 | -1.665 | 0.342 |
| H+ 0 - H+ 1 | before | 0.072 | | 0.074 | 0.961 | 0.772 |
| H 1 - H+ 1 | before | 0.370 | | 0.178 | 2.077 | 0.161 |
| Conditioning time | Batch |  |  | |  |  |  |
| after - before | 1 | 0.334 | | 0.103 | 3.237 | 0.001 |
| after - before | 2 | -0.136 | | 0.127 | -1.071 | 0.284 |
| **Model #4 : Behavioural response during session of the conditioning** | | | | | | |
| **Occurence of missed contact from human**  Treatment | |  |  |  | |  |
| H - H+  Trial number | Batch | | 0.812 | 0.271 - | 3.003 | | 0.003 |
| 1 - 2 | | -0.177 | 0.083 - | -2.118 | | 0.034 |

**Model #5 : Vocal response during session of the conditioning Trial number \* Treatment \* In prox. area Call duration (s) (log)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment \* In prox. area  H 0 - H+ 0 | - | 0.121 | 0.053 | 2.293 | 0.100 |
| H 0 - H 1 | - | 0.357 | 0.035 | 10.168 | <0.001 |
| H 0 - H+ 1 | - | 0.304 | 0.058 | 5.220 | <0.001 |
| H+ 0 - H 1 | - | 0.236 | 0.062 | 3.797 | <0.001 |
| H+ 0 - H+ 1 | - | 0.182 | 0.027 | 6.862 | <0.001 |
| H 1 - H+ 1 | - | -0.053 | 0.067 | -0.801 | 0.854 |
| Trial number | Batch  1 - 2 | - | -0.028 | 0.012 | -2.318 | 0.020 |
| Treatment | Batch  H - H+ | 1 | -0.097 | 0.074 | -1.307 | 0.191 |
| H - H+ | 2 | 0.165 | 0.077 | 2.155 | 0.031 |
| **-VocPC1** |  |  |  |  |  |
| Trial number \* In prox. area 0 - 1 | - | -0.050 | 0.028 | -1.804 | 0.071 |
| Treatment | Batch  H - H+ | 1 | 0.617 | 0.312 | 1.977 | 0.048 |
| H - H+ | 2 | -0.537 | 0.321 | -1.675 | 0.094 |
| In prox. area | Batch  0 - 1 | 1 | -0.291 | 0.113 | -2.568 | 0.010 |
| 0 - 1 | 2 | 0.184 | 0.154 | 1.191 | 0.234 |
| **VocPC2** |  |  |  |  |  |
| Treatment \* In prox. area H 0 - H+ 0 | - | -0.097 | 0.149 | -0.649 | 0.916 |
| H 0 - H 1 | - | -0.401 | 0.081 | -4.930 | <0.001 |
| H 0 - H+ 1 | - | -0.216 | 0.159 | -1.356 | 0.527 |
| H+ 0 - H 1 | - | -0.304 | 0.167 | -1.821 | 0.263 |
| H+ 0 - H+ 1 | - | -0.119 | 0.062 | -1.936 | 0.213 |
| H 1 - H+ 1 | - | 0.185 | 0.176 | 1.049 | 0.720 |
| Trial number \* In prox. area  0 - 1 | - | -0.036 | 0.016 | -2.343 | 0.019 |
| Trial number | Batch  1 - 2 | - | 0.056 | 0.019 | 2.874 | 0.004 |
| In prox. area \* HumanID  0 AH - 1 AH | - | -0.085 | 0.068 | -1.245 | 0.598 |
| 0 AH - 0 AV | - | 0.290 | 0.151 | 1.919 | 0.220 |
| 0 AH - 1 AV | - | -0.145 | 0.167 | -0.870 | 0.820 |
| 1 AH - 0 AV | - | 0.375 | 0.164 | 2.293 | 0.100 |
| 1 AH - 1 AV | - | -0.060 | 0.178 | -0.336 | 0.987 |
| 0 AV - 1 AV | - | -0.435 | 0.075 | -5.769 | <0.001 |
| **VocPC3** |  |  |  |  |  |
| Trial number \* HumanID AH - AV | - | -0.040 | 0.014 | -2.824 | 0.005 |

Table S3 : Table of estimates (standard error SE and 95% confidence intervals) from from models, computed using the ‘emmeans’ (categorical fixed effect) and ‘lstrends’ (continuous fixed effect) functions of the ‘Emmeans’ R package. Estimates indicated are either for groups or slopes, depending on the categorial or continuous variable (trial number). Estimates for all interpretable fixed effect, interacting or not and significant or nor are indicated.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **factor** | **first interaction (if**  **any)** | **second interaction (if**  **any)** | **estimate** | **SE** | **Lower 95%confidence**  **int.** | **Upper 95%confidence**  **int.** |
| **Model #1 : behavioural response during the Reunion of Isolation/Reunion test** | | | | | | |
| **ReuPC1** Treatment \* Conditioning  time |  |  | |  |  |  |
| H | after | 1.195 | | 0.220 | 0.759 | 1.632 |
| H+ | after | 1.173 | | 0.220 | 0.737 | 1.610 |
| H | before | -2.234 | | 0.220 | -2.671 | -1.798 |
| H+  Conditioning time | Batch | before | -0.134 | | 0.220 | -0.571 | 0.302 |
| after | 1 | 1.515 | | 0.222 | 1.074 | 1.956 |
| before | 1 | 0.069 | | 0.222 | -0.372 | 0.510 |
| after | 2 | 0.854 | | 0.222 | 0.413 | 1.295 |
| before Treatment \*  HumanID | 2 | -2.437 | | 0.222 | -2.878 | -1.996 |
| H | AH | -0.862 | | 0.240 | -1.344 | -0.380 |
| H+ | AH | 0.772 | | 0.240 | 0.290 | 1.254 |
| H | AV | -0.177 | | 0.240 | -0.659 | 0.305 |
| H+ | AV | 0.267 | | 0.240 | -0.215 | 0.749 |
| **ReuPC2 (sqrt+4)** |  |  | |  |  |  |
| Conditioning time | Batch |  |  | |  |  |  |
| after | 1 | 2.106 | | 0.073 | 1.962 | 2.250 |
| before | 1 | 1.970 | | 0.073 | 1.826 | 2.114 |
| after | 2 | 1.664 | | 0.073 | 1.520 | 1.808 |
| before | 2 | 2.080 | | 0.073 | 1.936 | 2.224 |
| Treatment |  |  | |  |  |  |
| H |  | 1.991 | | 0.051 | 1.889 | 2.093 |
| H+ |  | 1.919 | | 0.051 | 1.817 | 2.021 |
| HumanID |  |  | |  |  |  |
| AH |  | 1.903 | | 0.051 | 1.800 | 2.006 |
| AV |  | 2.007 | | 0.051 | 1.904 | 2.110 |
| **-ReuPC3**  **(sqrt +3)** |  |  | |  |  |  |
| Treatment \*  Conditioning time |  |  | |  |  |  |
| H | after | 1.712 | | 0.055 | 1.603 | 1.821 |
| H+ | after | 1.964 | | 0.055 | 1.855 | 2.073 |
| H | before | 1.525 | | 0.055 | 1.416 | 1.634 |
| H+  Treatment | Batch | before | 1.583 | | 0.055 | 1.474 | 1.692 |
| H | 1 | 1.539 | | 0.060 | 1.419 | 1.658 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| H+ | 1 | 1.853 | 0.060 | 1.733 | 1.972 |
| H | 2 | 1.699 | 0.060 | 1.579 | 1.819 |
| H+  Conditioning time | Batch | 2 | 1.695 | 0.060 | 1.575 | 1.814 |
| after | 1 | 1.898 | 0.056 | 1.788 | 2.008 |
| before | 1 | 1.494 | 0.056 | 1.383 | 1.604 |
| after | 2 | 1.779 | 0.056 | 1.668 | 1.889 |
| before | 2 | 1.615 | 0.056 | 1.505 | 1.725 |
| HumanID |  |  |  |  |  |
| AH |  | 1.678 | 0.042 | 1.593 | 1.762 |
| AV |  | 1.715 | 0.042 | 1.630 | 1.800 |

**Model #2 : Vocal response Isolation/Reunion tests : Treatment \* Phase \* Conditioning time**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Call duration**  **(s) (log)**  Conditioning time | Batch |  | | | | |
| after | 1 | -1.166 | 0.039 | -1.243 | -1.090 |
| before | 1 | -1.337 | 0.041 | -1.418 | -1.257 |
| after | 2 | -0.928 | 0.041 | -1.008 | -0.848 |
| before | 2 | -1.326 | 0.048 | -1.420 | -1.233 |
| Treatment |  |  |  |  |  |
| H |  | -1.125 | 0.035 | -1.194 | -1.056 |
| H+ |  | -1.254 | 0.033 | -1.320 | -1.189 |
| Phase |  |  |  |  |  |
| isolation |  | -1.063 | 0.030 | -1.122 | -1.003 |
| reunion H |  | -1.316 | 0.029 | -1.373 | -1.260 |
| HumanID |  |  |  |  |  |
| AH |  | -1.204 | 0.034 | -1.271 | -1.137 |
| AV |  | -1.175 | 0.035 | -1.243 | -1.107 |
| **-VocPC1** |  |  |  |  |  |
| Treatment |  |  |  |  |  |
| H |  | 0.436 | 0.197 | 0.050 | 0.821 |
| H+ |  | 0.687 | 0.186 | 0.323 | 1.051 |
| Phase |  |  |  |  |  |
| isolation |  | 0.341 | 0.161 | 0.025 | 0.656 |
| reunion H |  | 0.782 | 0.154 | 0.480 | 1.083 |
| HumanID |  |  |  |  |  |
| AH |  | 0.763 | 0.190 | 0.391 | 1.136 |
| AV |  | 0.359 | 0.193 | -0.020 | 0.738 |
| Batch |  |  |  |  |  |
| 1 |  | 1.605 | 0.183 | 1.247 | 1.963 |
| 2  Conditioning time |  | -0.482 | 0.202 | -0.877 | -0.087 |
| after |  | 0.594 | 0.167 | 0.268 | 0.921 |
| before |  | 0.528 | 0.185 | 0.166 | 0.890 |
| **VocPC2** |  |  |  |  |  |
| Conditioning |  |  |  |  |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| time | Batch |  |  |  |  |  |
| after | 1 | -0.645 | 0.129 | -0.898 | -0.392 |
| before | 1 | 0.187 | 0.133 | -0.073 | 0.448 |
| after | 2 | -1.290 | 0.137 | -1.558 | -1.022 |
| before | 2 | 0.465 | 0.150 | 0.172 | 0.759 |
| Treatment |  |  |  |  |  |
| H |  | -0.340 | 0.128 | -0.591 | -0.089 |
| H+ |  | -0.301 | 0.121 | -0.539 | -0.064 |
| Phase |  |  |  |  |  |
| isolation |  | -0.464 | 0.096 | -0.653 | -0.276 |
| reunion H |  | -0.177 | 0.094 | -0.361 | 0.007 |
| HumanID |  |  |  |  |  |
| AH |  | -0.097 | 0.125 | -0.342 | 0.147 |
| AV |  | -0.544 | 0.126 | -0.790 | -0.297 |
| **-VocPC3** |  |  |  |  |  |
| Conditioning time | Batch |  |  |  |  |  |
| after | 1 | -0.415 | 0.086 | -0.583 | -0.248 |
| before | 1 | -0.724 | 0.089 | -0.898 | -0.549 |
| after | 2 | 0.142 | 0.091 | -0.036 | 0.319 |
| before | 2 | 0.277 | 0.104 | 0.074 | 0.481 |
| Treatment |  |  |  |  |  |
| H |  | -0.068 | 0.077 | -0.219 | 0.083 |
| H+ |  | -0.292 | 0.073 | -0.434 | -0.149 |
| Phase |  |  |  |  |  |
| isolation |  | -0.107 | 0.060 | -0.225 | 0.010 |
| reunion H |  | -0.253 | 0.058 | -0.366 | -0.140 |
| HumanID |  |  |  |  |  |
| AH |  | -0.136 | 0.074 | -0.282 | 0.010 |
| AV |  | -0.224 | 0.076 | -0.372 | -0.076 |

**Model #3 : Vocal response during the Reunion of Isolation/Reunion tests : conditioning time \* Treatment \* In prox. area**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Call duration**  **(s) (log)**  Treatment \* In prox. area |  | | | | | |
| H | 0 | after | -1.154 | 0.052 | -1.256 | -1.053 |
| H+ | 0 | after | -1.194 | 0.049 | -1.289 | -1.099 |
| H | 1 | after | -1.277 | 0.056 | -1.386 | -1.167 |
| H+ | 1 | after | -1.244 | 0.052 | -1.345 | -1.142 |
| H | 0 | before | -1.317 | 0.059 | -1.434 | -1.201 |
| H+ | 0 | before | -1.505 | 0.052 | -1.607 | -1.402 |
| H | 1 | before | -1.629 | 0.071 | -1.768 | -1.490 |
| H+  Conditioning time | Batch | 1 | before | -1.571 | 0.054 | -1.677 | -1.465 |
| after | 1 |  | -1.373 | 0.050 | -1.471 | -1.276 |
| before | 1 |  | -1.458 | 0.050 | -1.556 | -1.360 |
| after | 2 |  | -1.061 | 0.051 | -1.162 | -0.961 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| before | 2 |  | -1.553 | 0.062 | -1.674 | -1.433 |
| HumanID |  |  |  |  |  |  |
| AH |  |  | -1.412 | 0.045 | -1.501 | -1.324 |
| AV |  |  | -1.311 | 0.046 | -1.402 | -1.220 |
| **-VocPC1** |  |  |  |  |  |  |
| Treatment \* In prox. area | conditioning time |  |  |  |  |  |  |
| H | 0 | after | 0.718 | 0.303 | 0.124 | 1.312 |
| H+ | 0 | after | 1.063 | 0.285 | 0.505 | 1.622 |
| H | 1 | after | 0.945 | 0.330 | 0.298 | 1.592 |
| H+ | 1 | after | 0.842 | 0.306 | 0.242 | 1.442 |
| H | 0 | before | 0.745 | 0.360 | 0.039 | 1.450 |
| H+ | 0 | before | 0.796 | 0.312 | 0.185 | 1.408 |
| H | 1 | before | 1.758 | 0.433 | 0.911 | 2.606 |
| H+  Conditioning time | Batch | 1 | before | 0.434 | 0.322 | -0.198 | 1.066 |
| after | 1 |  | 2.072 | 0.293 | 1.498 | 2.646 |
| before | 1 |  | 1.520 | 0.294 | 0.944 | 2.096 |
| after | 2 |  | -0.288 | 0.300 | -0.875 | 0.299 |
| before | 2 |  | 0.347 | 0.375 | -0.389 | 1.082 |
| HumanID |  |  |  |  |  |  |
| AH |  |  | 1.145 | 0.228 | 0.699 | 1.592 |
| AV |  |  | 0.680 | 0.237 | 0.216 | 1.144 |

**VocPC2\***

\*Note : due to a three way interaction close to significance level, contrasts were generating with the three way interaction or with the two ways interactions of interests

Treatment \* In prox. area | conditioning time

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| H | 0 |  | after | -0.773 | 0.157 | -1.081 | -0.465 |
| H+ | 0 |  | after | -0.884 | 0.147 | -1.173 | -0.596 |
| H | 1 |  | after | -0.557 | 0.170 | -0.891 | -0.223 |
| H+ | 1 |  | after | -0.750 | 0.158 | -1.059 | -0.441 |
| H | 0 |  | before | 0.312 | 0.180 | -0.040 | 0.664 |
| H+ | 0 |  | before | 0.328 | 0.159 | 0.016 | 0.640 |
| H | 1 |  | before | 1.110 | 0.216 | 0.687 | 1.534 |
| H+  In prox. area | Conditioning  time | 1 |  | before | 0.663 | 0.164 | 0.342 | 0.984 |
| 0 | after |  |  | 0.891 | 0.208 | 0.483 | 1.298 |
| 1 | after |  |  | 0.893 | 0.225 | 0.452 | 1.335 |
| 0 | before |  |  | 0.771 | 0.240 | 0.300 | 1.241 |
| 1  Treatment \* In prox. area | before |  |  | 1.096 | 0.274 | 0.558 | 1.634 |
| H |  | 0 |  | -0.230 | 0.147 | -0.518 | 0.057 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| H+ |  | 0 |  | -0.278 | 0.134 | -0.542 | -0.015 |
| H |  | 1 |  | 0.277 | 0.162 | -0.042 | 0.595 |
| H+  In prox. area | Batch |  | 1 |  | -0.044 | 0.139 | -0.316 | 0.229 |
| 0 | 1 |  |  | -0.136 | 0.135 | -0.400 | 0.128 |
| 1 | 1 |  |  | 0.087 | 0.141 | -0.189 | 0.363 |
| 0 | 2 |  |  | -0.372 | 0.149 | -0.664 | -0.081 |
| 1  Conditioning time | Batch | 2 |  |  | 0.146 | 0.162 | -0.171 | 0.463 |
| after | 1 |  |  | -0.365 | 0.151 | -0.660 | -0.069 |
| before | 1 |  |  | 0.315 | 0.152 | 0.018 | 0.613 |
| after | 2 |  |  | -1.117 | 0.156 | -1.424 | -0.811 |
| before | 2 |  |  | 0.891 | 0.187 | 0.526 | 1.257 |
| HumanID |  |  |  |  |  |  |  |
| AH |  |  |  | 0.154 | 0.139 | -0.119 | 0.427 |
| AV |  |  |  | -0.292 | 0.143 | -0.571 | -0.012 |
| **-VocPC3** |  |  |  |  |  |  |  |
| Treatment \* In prox. area |  |  |  |  |  |  |  |
| H | 0 |  | after | -0.022 | 0.096 | -0.211 | 0.166 |
| H+ | 0 |  | after | -0.249 | 0.090 | -0.426 | -0.073 |
| H | 1 |  | after | -0.147 | 0.109 | -0.361 | 0.067 |
| H+ | 1 |  | after | -0.347 | 0.100 | -0.543 | -0.150 |
| H | 0 |  | before | -0.322 | 0.116 | -0.551 | -0.094 |
| H+ | 0 |  | before | -0.356 | 0.101 | -0.554 | -0.157 |
| H | 1 |  | before | -0.057 | 0.150 | -0.350 | 0.236 |
| H+  Conditioning time | Batch | 1 |  | before | -0.427 | 0.105 | -0.634 | -0.221 |
| after | 1 |  |  | -0.483 | 0.092 | -0.664 | -0.303 |
| before | 1 |  |  | -0.818 | 0.094 | -1.001 | -0.634 |
| after | 2 |  |  | 0.100 | 0.095 | -0.086 | 0.287 |
| before | 2 |  |  | 0.236 | 0.122 | -0.002 | 0.475 |
| HumanID |  |  |  |  |  |  |  |
| AH |  |  |  | -0.190 | 0.081 | -0.349 | -0.032 |
| AV |  |  |  | -0.292 | 0.084 | -0.456 | -0.127 |

**Model #4 : Behavioural response during sessions of the conditioning**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **CondPC1**  Trial number |  | | | |
| - | 0.2 | 0.03 | 0.15 | 0.25 |
| Treatment |  |  |  |  |
| H | -0.080 | 0.240 | -0.562 | 0.402 |
| H+ | 0.064 | 0.242 | -0.422 | 0.550 |
| HumanID |  |  |  |  |
| AH | -0.206 | 0.243 | -0.693 | 0.281 |
| AV | 0.190 | 0.245 | -0.300 | 0.681 |
| Batch |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1 | 0.658 | 0.243 | 0.171 | 1.145 |
| 2 | -0.674 | 0.245 | -1.165 | -0.183 |
| **CondPC2** |  |  |  |  |
| Trial number  - | -0.17 | 0.02 | -0.22 | -0.12 |
| Treatment  H | 0.385 | 0.152 | 0.080 | 0.690 |
| H+ | -0.363 | 0.154 | -0.672 | -0.054 |
| HumanID  AH | 0.061 | 0.153 | -0.246 | 0.368 |
| AV | -0.039 | 0.156 | -0.352 | 0.273 |
| Batch  1 | -0.312 | 0.153 | -0.619 | -0.004 |
| 2 | 0.333 | 0.156 | 0.021 | 0.646 |
| **CondPC3** |  |  |  |  |
| Trial number  - | -0.001 | 0.017 | -0.035 | 0.032 |
| Treatment  H | -0.176 | 0.103 | -0.383 | 0.031 |
| H+ | 0.188 | 0.105 | -0.022 | 0.398 |
| HumanID  AH | -0.043 | 0.104 | -0.252 | 0.165 |
| AV | 0.055 | 0.106 | -0.157 | 0.267 |
| Batch  1 | 0.010 | 0.104 | -0.198 | 0.219 |
| 2  **Occurence of missed contact from human**  Treatment  H - | 0.002  -0.110 | 0.106  0.188 | -0.210  -0.479 | 0.214  0.259 |
| H+ - | -0.922 | 0.206 | -1.325 | -0.519 |
| Trial | Batch  1 2 to 11 | -0.303 | 0.062 | -0.425 | -0.182 |
| 2 2 to 11 | -0.127 | 0.059 | -0.243 | -0.010 |

**Model #5 : Vocal response during session of the conditioning Trial number \* Treatment \* In prox. area**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Call duration**  **(s) (log)**  Treatment \* In prox. area |  | | | | |
| H | 0 | -1.263 | 0.038 | -1.338 | -1.189 |
| H+ | 0 | -1.385 | 0.037 | -1.457 | -1.313 |
| H | 1 | -1.621 | 0.050 | -1.719 | -1.522 |
| H+  Trial number | Batch | 1 | -1.567 | 0.044 | -1.654 | -1.481 |
| 1 |  | -0.053 | 0.009 | -0.070 | -0.036 |
| 2  Treatment | Batch |  | -0.025 | 0.009 | -0.042 | -0.008 |
| H | 1 | -1.621 | 0.053 | -1.724 | -1.517 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| H+ | 1 | -1.523 | 0.055 | -1.631 | -1.416 |
| H | 2 | -1.264 | 0.060 | -1.381 | -1.146 |
| H+ | 2 | -1.429 | 0.052 | -1.532 | -1.326 |
| HumanID |  |  |  |  |  |
| AH |  | -0.046 | 0.009 | -0.063 | -0.029 |
| AV |  | -0.032 | 0.009 | -0.050 | -0.015 |
| **-VocPC1** |  |  |  |  |  |
| Trial number \* In prox. area |  |  |  |  |  |
| 0 |  | 0.058 | 0.018 | 0.023 | 0.094 |
| 1  Treatment | Batch |  | 0.109 | 0.031 | 0.048 | 0.169 |
| H | 1 | -0.053 | 0.219 | -0.481 | 0.375 |
| H+ | 1 | -0.670 | 0.227 | -1.115 | -0.225 |
| H | 2 | -2.570 | 0.248 | -3.056 | -2.084 |
| H+  In prox. area | Batch | 2 | -2.033 | 0.218 | -2.460 | -1.606 |
| 0 | 1 | -0.507 | 0.153 | -0.807 | -0.207 |
| 1 | 1 | -0.216 | 0.183 | -0.575 | 0.144 |
| 0 | 2 | -2.210 | 0.155 | -2.513 | -1.906 |
| 1 | 2 | -2.393 | 0.213 | -2.811 | -1.975 |
| HumanID |  |  |  |  |  |
| AH |  | 0.091 | 0.027 | 0.037 | 0.144 |
| AV |  | 0.076 | 0.028 | 0.021 | 0.132 |
| **VocPC2** |  |  |  |  |  |
| Treatment \* In prox. area |  |  |  |  |  |
| H+ | 1 | -1.372 | 0.181 | -1.727 | -1.018 |
| H | 0 | 0.124 | 0.107 | -0.085 | 0.333 |
| H+ | 0 | 0.220 | 0.104 | 0.016 | 0.425 |
| H | 1 | 0.525 | 0.130 | 0.269 | 0.780 |
| H+  Trial number \* In prox. area | 1 | 0.340 | 0.119 | 0.108 | 0.572 |
| 0 |  | -0.091 | 0.010 | -0.110 | -0.072 |
| 1  Trial number | Batch |  | -0.054 | 0.017 | -0.088 | -0.021 |
| 1 |  | -0.045 | 0.015 | -0.074 | -0.015 |
| 2  In prox. area \* HumanID |  | -0.100 | 0.015 | -0.130 | -0.071 |
| 0 | AH | 0.317 | 0.106 | 0.110 | 0.524 |
| 1 | AH | 0.402 | 0.123 | 0.161 | 0.643 |
| 0 | AV | 0.027 | 0.107 | -0.182 | 0.236 |
| 1 | AV | 0.462 | 0.128 | 0.212 | 0.712 |
| **VocPC3** |  |  |  |  |  |
| Trial number \* HumanID |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| AH | -0.048 | 0.011 | -0.070 | -0.026 |
| AV | -0.007 | 0.012 | -0.031 | 0.016 |
| Treatment |  |  |  |  |
| H | 0.193 | 0.082 | 0.033 | 0.353 |
| H+ | 0.121 | 0.076 | -0.029 | 0.270 |
| In prox. area |  |  |  |  |
| 0 | 0.205 | 0.052 | 0.103 | 0.308 |
| 1 | 0.108 | 0.069 | -0.026 | 0.243 |
| Batch |  |  |  |  |
| 1 | -0.181 | 0.077 | -0.333 | -0.030 |
| 2 | 0.495 | 0.083 | 0.332 | 0.659 |

Table S4 : Table of raw values of acoustic parameters in each comparison group of interest.

**Comparison of Isolation Reunion (Isolation/Reunion test : static and silent human)**

**Effect of proximity during reunion (Isolation/Reunion test, static and silent human)**

**Effect of proximity sessions of additional positive contacts (conditioning, interacting human)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Condi**  **tionin Treat Phas g time ment e** | **Cond itioni ng**  **Ncalls time** | **Trea tmen t** | **In prox. area** |  | **Ncalls** | **Time in conditi oning** | **Treat ment** | **In prox. area** | **Ncalls** |
| **Number of calls per group** |  |  |  |  |  |  |  |  |  |
| after H iso | 1204 after | H |  | 0 | 1482 | early | H | 0 | 1240 |
| reuH | 1976 |  |  | 1 | 484 |  |  | 1 | 164 |
| H+ iso | 1015 | H+ |  | 0 | 1531 |  | H+ | 0 | 1692 |
| reuH | 2163 |  |  | 1 | 568 |  |  | 1 | 222 |
| before H iso | 842 before | H | 0 | | 432 late | | H | 0 | 779 |
| reuH | 662 |  | 1 | | 226 | |  | 1 | 77 |
| H+ iso | 630 | H+ | 0 | | 609 | | H+ | 0 | 865 |

reuH 1251 1 706 1 129

**Mean**

**Mean of paramete r**

**Sd of paramete r**

**of parame ter**

**Sd of parame ter**

**Mean of paramet er**

**Sd of parame ter**

**Mean Dominant Frequency (kHz)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| after H iso | 0.304 | 0.071 after | H | 0 | 0.320 | 0.087 early | H | 0 | 0.314 | 0.039 |
| reuH | 0.324 | 0.092 |  | 1 | 0.337 | 0.105 |  | 1 | 0.327 | 0.036 |
| H+ iso | 0.302 | 0.064 | H+ | 0 | 0.314 | 0.086 | H+ | 0 | 0.322 | 0.041 |
| reuH | 0.320 | 0.093 |  | 1 | 0.335 | 0.103 |  | 1 | 0.329 | 0.040 |
| before H iso | 0.322 | 0.065 before | H | 0 | 0.334 | 0.080 late | H | 0 | 0.303 | 0.037 |
| reuH | 0.350 | 0.098 |  | 1 | 0.381 | 0.120 |  | 1 | 0.324 | 0.039 |
| H+ iso | 0.342 | 0.073 | H+ | 0 | 0.337 | 0.068 | H+ | 0 | 0.299 | 0.035 |
| reuH | 0.343 | 0.065 |  | 1 | 0.348 | 0.060 |  | 1 | 0.331 | 0.057 |
| **Min F peak (kHz)** |  |  |  |  |  |  |  |  |  |  |
| after H iso | 0.286 | 0.049 after | H | 0 | 0.296 | 0.046 early | H | 0 | 0.309 | 0.052 |
| reuH | 0.299 | 0.050 |  | 1 | 0.308 | 0.061 |  | 1 | 0.325 | 0.050 |
| H+ iso | 0.288 | 0.053 | H+ | 0 | 0.288 | 0.057 | H+ | 0 | 0.322 | 0.052 |
| reuH | 0.293 | 0.058 |  | 1 | 0.306 | 0.058 |  | 1 | 0.324 | 0.050 |
| before H iso | 0.315 | 0.051 before | H | 0 | 0.327 | 0.063 late | H | 0 | 0.296 | 0.045 |

after H

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | reuH | 0.333 | 0.062 |  | 1 | 0.345 | 0.059 |  | 1 | 0.316 | 0.040 |
| H+ | iso | 0.334 | 0.052 | H+ | 0 | 0.330 | 0.052 | H+ | 0 | 0.296 | 0.045 |
|  | reuH | 0.336 | 0.049 |  | 1 | 0.342 | 0.043 |  | 1 | 0.318 | 0.047 |
| **Mac F peak (kHz)** | | | | | | | | | | | |
| after H iso | | 0.931 | 1.071 after | H | 0 | 1.151 | 1.342 early | H | 0 | 0.731 | 0.892 |
| reuH | | 1.177 | 1.383 |  | 1 | 1.261 | 1.499 |  | 1 | 0.979 | 1.070 |
| H+ iso | | 0.821 | 1.068 | H+ | 0 | 1.045 | 1.284 | H+ | 0 | 0.677 | 0.750 |
| reuH | | 1.058 | 1.282 |  | 1 | 1.054 | 1.282 |  | 1 | 0.827 | 0.916 |
| before H iso | | 0.969 | 1.233 before | H | 0 | 0.911 | 1.161 late | H | 0 | 0.804 | 0.975 |
| reuH | | 1.080 | 1.346 |  | 1 | 1.419 | 1.600 |  | 1 | 1.013 | 1.121 |
| H+ iso | | 0.794 | 1.005 | H+ | 0 | 0.874 | 1.163 | H+ | 0 | 0.788 | 0.886 |
| reuH | | 0.844 | 1.136 |  | 1 | 0.786 | 1.060 |  | 1 | 1.040 | 1.070 |
| **Mode (Hz)** |  |  |  |  |  |  |  |  |  |  |  |
| after H iso | | 291.278 | 59.014 after | H | 0 | 302.674 | 51.629 early | H | 0 | 322.410 | 49.159 |
| reuH | | 305.321 | 52.397 |  | 1 | 313.777 | 54.076 |  | 1 | 340.177 | 42.013 |
| H+ iso | | 292.890 | 45.872 | H+ | 0 | 295.295 | 59.358 | H+ | 0 | 332.720 | 49.270 |
| reuH | | 301.645 | 67.420 |  | 1 | 317.938 | 84.665 |  | 1 | 336.006 | 50.457 |
| before H iso | | 321.339 | 50.148 before | H | 0 | 335.165 | 72.839 late | H | 0 | 303.054 | 45.965 |
|  | reuH | 346.629 | 92.602 |  | 1 | 368.842 | 119.373 |  | 1 | 322.763 | 40.934 |
| H+ | iso | 340.806 | 53.094 | H+ | 0 | 335.019 | 47.114 | H+ | 0 | 303.363 | 45.433 |
|  | reuH | 342.822 | 49.077 |  | 1 | 350.652 | 47.117 |  | 1 | 326.297 | 45.992 |
| **Mean (Hz)** |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | 1885.08 |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 1817.653 | 385.617 | 0 | 7 | 426.846 | 0 | 1494.859 | 294.318 |
|  |  |  |  | 1868.69 |  |  |  |  |
| reuH | 1879.520 | 423.215 | 1 | 3 | 410.882 | 1 | 1628.225 | 304.585 |

after H

early H

H+ H+

1887.65 H+

before H

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 1842.219 | 428.577 | 0 | 6 | 457.439 | 0 | 1443.195 | 263.108 |
|  |  |  |  | 1837.23 |  |  |  |  |
| reuH | 1878.859 | 453.032 | 1 | 2 | 442.185 | 1 | 1472.662 | 285.439 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | | | 1687.113 | 390.208 |  | 0 | 1 | 445.991 |  | 0 | 1507.883 | 269.191 |
|  | | |  |  |  |  | 1736.17 |  |  |  |  |  |
| reuH | | | 1786.125 | 457.160 |  | 1 | 6 | 462.373 |  | 1 | 1563.601 | 289.547 |
| **Q50 (Hz)** | | |  |  |  |  |  |  |  |  |  |  |
| after H iso | | | 750.232 | 484.174 after | H | 0 | 847.308 | 569.306 early | H | 0 | 493.711 | 212.211 |
| reuH | | | 842.007 | 554.088 |  | 1 | 831.133 | 505.754 |  | 1 | 567.891 | 232.048 |
| H+ iso | | | 778.814 | 545.853 | H+ | 0 | 865.811 | 614.602 | H+ | 0 | 473.187 | 152.404 |
| reuH | | | 858.754 | 600.966 |  | 1 | 813.264 | 558.239 |  | 1 | 494.802 | 159.335 |
| before H iso | | | 751.721 | 524.095 before | H | 0 | 766.585 | 539.701 late | H | 0 | 500.546 | 223.284 |
| reuH | | | 803.559 | 544.105 |  | 1 | 881.844 | 547.431 |  | 1 | 581.590 | 319.693 |
| H+ iso | | | 665.355 | 454.203 | H+ | 0 | 755.510 | 511.427 | H+ | 0 | 496.292 | 213.039 |
| reuH | | | 742.369 | 506.089 |  | 1 | 709.311 | 487.096 |  | 1 | 555.141 | 238.753 |
| **Q25 (Hz)** | | |  |  |  |  |  |  |  |  |  |  |
| after | H | iso | 302.742 | 69.646 after | H | 0 | 330.530 | 103.016 early | H | 0 | 287.444 | 46.594 |
|  |  | reuH | 330.573 | 101.286 |  | 1 | 331.723 | 96.435 |  | 1 | 309.890 | 50.641 |
|  | H+ | iso | 304.540 | 75.870 | H+ | 0 | 324.614 | 99.783 | H+ | 0 | 290.177 | 45.347 |
|  |  | reuH | 328.360 | 100.583 |  | 1 | 333.044 | 95.537 |  | 1 | 301.554 | 51.488 |

before H

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 1769.750 | 442.237 | 0 | 2 | 443.617 | 0 | 1524.356 | 294.879 |
|  |  |  |  | 1851.11 |  |  |  |  |
| reuH | 1822.634 | 433.496 | 1 | 7 | 414.251 | 1 | 1581.281 | 341.346 |

1811.10

late H

H+ H+

1812.03 H+

after H

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| before H | iso | 317.037 | 82.577 before | H | 0 | 328.490 | 95.369 late | H | 0 | 283.892 | 41.855 |
|  | reuH | 345.858 | 104.483 |  | 1 | 380.512 | 112.884 |  | 1 | 320.480 | 64.909 |
| H+ | iso | 324.410 | 81.132 | H+ | 0 | 333.849 | 77.673 | H+ | 0 | 281.491 | 39.744 |
|  | reuH | 335.275 | 74.787 |  | 1 | 333.792 | 70.761 |  | 1 | 307.556 | 57.016 |
| **Q75 (Hz)** |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | 2855.25 |  |  |  |  |  |

iso 2724.508 901.531

after H

0 1 962.026

2800.45

early H

0 1907.359 821.008

reuH 2838.172 964.096 1 4 966.843 1 2271.884 873.368

H+ iso 2797.501 989.550 H+

2871.37

0 8 1036.874 H+

2731.68

0 1795.251 756.385

reuH 2841.678 1032.082 1 2 1032.696 1 1886.704 797.162

before H

iso 2667.213 1043.411

before H

2718.41

0 7 1054.461

2724.73

late H

0 2005.637 806.336

reuH 2716.523 1026.942 1 0 979.535 1 2132.724 873.945

H+ iso 2483.126 934.606 H+

2770.86

0 2 1082.494 H+

2509.77

0 2019.569 718.732

**Centroid (Hz)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 1817.653 | 385.617 | 0 | 7 | 426.846 | 0 | 1494.859 | 294.318 |
|  |  |  |  | 1868.69 |  |  |  |  |
| reuH | 1879.520 | 423.215 | 1 | 3 | 410.882 | 1 | 1628.225 | 304.585 |

reuH 2669.632 1139.513 1

8 1182.463 1 2167.271 749.616

after H

after H

1885.08

early H

H+ H+

1887.65 H+

before H

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 1842.219 | 428.577 | 0 | 6 | 457.439 | 0 | 1443.195 | 263.108 |
|  |  |  |  | 1837.23 |  |  |  |  |
| reuH | 1878.859 | 453.032 | 1 | 2 | 442.185 | 1 | 1472.662 | 285.439 |

before H

1811.10

late H

H+ iso 1687.113 390.208 H+

1812.03

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 1769.750 | 442.237 | 0 | 2 | 443.617 | 0 | 1524.356 | 294.879 |
|  |  |  |  | 1851.11 |  |  |  |  |
| reuH | 1822.634 | 433.496 | 1 | 7 | 414.251 | 1 | 1581.281 | 341.346 |

0 1 445.991 H+

1736.17

0 1507.883 269.191

**Sd (Hz)**

after H

reuH 1786.125 457.160 1

6 462.373 1 1563.601 289.547

2145.79

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 2134.495 | 176.184 after H | 0 | 5 | 174.256 early H | 0 | 1990.877 | 174.232 |
|  |  |  |  | 2141.37 |  |  |  |  |
| reuH | 2144.085 | 175.255 | 1 | 2 | 177.553 | 1 | 2047.922 | 175.263 |

H+ H+

2147.76 H+

before H

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 2153.259 | 199.831 | 0 | 1 | 186.968 | 0 | 1942.072 | 169.828 |
|  |  |  |  | 2120.01 |  |  |  |  |
| reuH | 2140.791 | 187.566 | 1 | 9 | 192.350 | 1 | 1940.150 | 184.449 |

before H

2111.66

late H

H+ iso 2022.945 173.442 H+

2106.11

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 2069.712 | 201.094 | 0 | 0 | 193.862 | 0 | 2007.860 | 175.699 |
|  |  |  |  | 2070.05 |  |  |  |  |
| reuH | 2096.807 | 194.727 | 1 | 4 | 195.140 | 1 | 1992.677 | 170.148 |

0 4 205.720 H+

2071.60

0 1974.469 164.606

**IQR (Hz)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| iso | 2421.766 | 863.329 | 0 | 0 | 902.648 | 0 | 1619.915 | 797.974 |
| reuH | 2507.599 | 906.090 | 1 | 2468.73 | 912.873 | 1 | 1961.994 | 849.624 |

reuH 2095.007 211.699 1

1 217.408 1 1964.886 163.211

after H

after H

2524.72

early H

H+ iso 2492.962 947.609 H+

1

2546.76

0 3 981.693 H+

2398.63

0 1505.074 739.851

reuH 2513.318 978.741 1 8 986.570 1 1585.150 779.768

before H

iso 2350.176 993.024

before H

2389.92

0 7 1001.070

2344.21

late H

0 1721.745 785.012

reuH 2370.665 976.677 1 9 935.061 1 1812.245 828.439

H+ iso 2158.716 893.869 H+

2437.01

0 3 1036.722 H+

2175.98

0 1738.078 702.471

**Sfm**

reuH 2334.358 1094.079 1

6 1135.706 1 1859.715 720.425

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| after H iso | 0.545 | 0.112 after | H | 0 | 0.565 | 0.121 early | H | 0 | 0.447 | 0.091 |
| reuH | 0.563 | 0.120 |  | 1 | 0.560 | 0.117 |  | 1 | 0.485 | 0.095 |
| H+ iso | 0.551 | 0.121 | H+ | 0 | 0.564 | 0.129 | H+ | 0 | 0.432 | 0.083 |
| reuH | 0.562 | 0.129 |  | 1 | 0.549 | 0.127 |  | 1 | 0.437 | 0.089 |
| before H iso | 0.531 | 0.129 before | H | 0 | 0.541 | 0.131 late | H | 0 | 0.454 | 0.088 |
| reuH | 0.544 | 0.128 |  | 1 | 0.552 | 0.123 |  | 1 | 0.468 | 0.104 |
| H+ iso | 0.506 | 0.115 | H+ | 0 | 0.538 | 0.130 | H+ | 0 | 0.454 | 0.085 |
| reuH | 0.530 | 0.134 |  | 1 | 0.515 | 0.136 |  | 1 | 0.467 | 0.090 |
| **Sh** |  |  |  |  |  |  |  |  |  |  |
| after H iso | 0.810 | 0.064 after | H | 0 | 0.820 | 0.068 early | H | 0 | 0.757 | 0.058 |
| reuH | 0.820 | 0.067 |  | 1 | 0.821 | 0.065 |  | 1 | 0.781 | 0.056 |
| H+ iso | 0.810 | 0.068 | H+ | 0 | 0.817 | 0.072 | H+ | 0 | 0.751 | 0.052 |
| reuH | 0.816 | 0.072 |  | 1 | 0.811 | 0.070 |  | 1 | 0.756 | 0.054 |
| before H iso | 0.802 | 0.071 before | H | 0 | 0.804 | 0.073 late | H | 0 | 0.761 | 0.056 |
| reuH | 0.810 | 0.071 |  | 1 | 0.824 | 0.064 |  | 1 | 0.775 | 0.062 |
| H+ iso | 0.788 | 0.064 | H+ | 0 | 0.803 | 0.071 | H+ | 0 | 0.762 | 0.055 |
| reuH | 0.797 | 0.074 |  | 1 | 0.789 | 0.075 |  | 1 | 0.775 | 0.056 |
| **Entropy H** |  |  |  |  |  |  |  |  |  |  |
| after H iso | 0.626 | 0.049 after | H | 0 | 0.625 | 0.049 early | H | 0 | 0.595 | 0.044 |
| reuH | 0.624 | 0.049 |  | 1 | 0.624 | 0.049 |  | 1 | 0.607 | 0.044 |
| H+ iso | 0.624 | 0.051 | H+ | 0 | 0.622 | 0.054 | H+ | 0 | 0.589 | 0.040 |
| reuH | 0.621 | 0.054 |  | 1 | 0.614 | 0.053 |  | 1 | 0.592 | 0.042 |
| before H iso | 0.613 | 0.050 before | H | 0 | 0.613 | 0.053 late | H | 0 | 0.595 | 0.042 |
| reuH | 0.614 | 0.051 |  | 1 | 0.618 | 0.047 |  | 1 | 0.604 | 0.046 |
| H+ iso | 0.602 | 0.047 | H+ | 0 | 0.603 | 0.053 | H+ | 0 | 0.595 | 0.042 |
| reuH | 0.597 | 0.056 |  | 1 | 0.589 | 0.057 |  | 1 | 0.602 | 0.044 |
| **Skewness** |  |  |  |  |  |  |  |  |  |  |
| after H iso | 4.525 | 0.678 after | H | 0 | 4.485 | 0.663 early | H | 0 | 4.439 | 0.560 |
| reuH | 4.442 | 0.690 |  | 1 | 4.302 | 0.754 |  | 1 | 4.405 | 0.562 |
| H+ iso | 4.637 | 0.598 | H+ | 0 | 4.592 | 0.669 | H+ | 0 | 4.467 | 0.535 |
| reuH | 4.557 | 0.689 |  | 1 | 4.516 | 0.721 |  | 1 | 4.417 | 0.578 |
| before H iso | 4.408 | 0.556 before | H | 0 | 4.473 | 0.578 late | H | 0 | 4.587 | 0.501 |
| reuH | 4.320 | 0.743 |  | 1 | 4.017 | 0.915 |  | 1 | 4.443 | 0.638 |
| H+ iso | 4.527 | 0.528 | H+ | 0 | 4.510 | 0.533 | H+ | 0 | 4.630 | 0.504 |
| reuH | 4.549 | 0.545 |  | 1 | 4.579 | 0.543 |  | 1 | 4.488 | 0.594 |
| **Kurtosis** |  |  |  |  |  |  |  |  |  |  |
| after H iso | 24.851 | 6.106 after | H | 0 | 24.571 | 5.721 early | H | 0 | 23.585 | 5.285 |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | reuH | 24.187 | 5.944 |  | 1 | 22.948 | 6.454 |  | 1 | 23.511 | 5.443 |
| H+ | iso | 25.901 | 5.610 | H+ | 0 | 25.663 | 5.917 | H+ | 0 | 23.892 | 5.198 |
|  | reuH | 25.329 | 6.093 |  | 1 | 24.865 | 6.429 |  | 1 | 23.557 | 5.393 |
| before H | iso | 23.361 | 5.093 before | H | 0 | 24.071 | 5.215 late | H | 0 | 25.056 | 4.700 |
|  | reuH | 22.971 | 6.224 |  | 1 | 20.784 | 7.356 |  | 1 | 24.180 | 5.847 |
| H+ | iso | 24.464 | 5.004 | H+ | 0 | 24.275 | 4.880 | H+ | 0 | 25.495 | 4.883 |
|  | reuH | 24.699 | 5.008 |  | 1 | 25.036 | 5.006 |  | 1 | 24.380 | 5.485 |
| **Call duration (s)** | | | | | | | | | | | |
| after H iso | | 0.497 | 0.252 after | H | 0 | 0.366 | 0.193 early | H | 0 | 0.358 | 0.178 |
| reuH | | 0.366 | 0.205 |  | 1 | 0.364 | 0.236 |  | 1 | 0.243 | 0.166 |
| H+ iso | | 0.435 | 0.164 | H+ | 0 | 0.349 | 0.163 | H+ | 0 | 0.330 | 0.174 |
| reuH | | 0.339 | 0.161 |  | 1 | 0.316 | 0.156 |  | 1 | 0.265 | 0.151 |
| before H | iso | 0.387 | 0.195 before H | | 0 | 0.326 | 0.177 late | H | 0 | 0.301 | 0.185 |
|  | reuH | 0.308 | 0.180 | | 1 | 0.272 | 0.181 |  | 1 | 0.236 | 0.177 |
| H+ | iso | 0.329 | 0.134 H+ | | 0 | 0.262 | 0.122 | H+ | 0 | 0.255 | 0.134 |
|  | reuH | 0.248 | 0.123 | | 1 | 0.231 | 0.120 |  | 1 | 0.203 | 0.111 |

Table S5: Number of calls of each call type recorded during the session and the number of pigs involved in the count. Taking into account the different statistical variable that needed to be add in the models, and thus the number of calls and pigs needed to have reliable statistical analysis, it was thus decided to use only grunts in this study.

**Before conditioning (Isolation/Reunion test – Reunion with H)**

**During conditioning (all trials pooled)**

**After conditioning (Isolation/Reunion test – Reunion with H)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Call type** | **Treatment** | **N calls** | **N pigs** |  | **N calls N pigs** | **N calls** | **N pigs** |  |
| bark | H | 14 |  | 5 | 13 7 |  | 6 | 3 |
| grunt | H | 670 |  | 21 | 3979 29 |  | 1981 | 25 |
| mixed | H | 8 |  | 1 | 172 12 |  | 157 | 9 |
| scream | H | 0 |  | 0 | 14 4 |  | 39 | 2 |
| squeal | H | 11 |  | 2 | 94 10 |  | 66 | 11 |
| bark | H+ | 4 |  | 2 | 18 6 |  | 1 | 1 |
| grunt | H+ | 1244 |  | 27 | 5006 29 |  | 2072 | 27 |
| mixed | H+ | 0 |  | 0 | 142 12 |  | 21 | 3 |
| scream | H+ | 0 |  | 0 | 7 2 |  | 0 | 0 |
| squeal | H+ | 8 |  | 2 | 50 8 |  | 25 | 6 |