Manuscript v1: https://www.biorxiv.org/content/10.1101/2022.03.15.484457v1.full.pdf

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

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.

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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__How_to_cite_Praat.html)

Yes. See line 210 "Praat software (Boersma and Paul 2001), version 6.0 from 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-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). 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)."

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

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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.

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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.

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

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

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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]. 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). 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.

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

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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 (χ^2_1 = 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 (χ^2_1 = 10.3, p = 0.001) on the one hand, and between the location and the treatment (χ^2_1 = 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

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. 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

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.

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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.

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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.

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

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 (C) 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.

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*

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

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, $\chi^2_1 = 6.01$, $p = 0.01$) but not the others (ReuPC2 and ReuPC3 ($\chi^2_1 < 1.98$, $p > 0.16$, table S1)." Line 496

- "These interacting effects of the human identity and treatment on behaviour were not found when considering the reunions of the conditioning ($\chi^2_1 < 1.32$, $p > 0.25$ for all CondPCs, table S1)." Line 501

- "Interactions between the human identity and conditioning time were not significant, neither considering the reunions of the Isolation/Reunion test (ReuPCs, $\chi^2_1 < 0.642$, $p > 0.42$, tables S1), neither the trial number during the session of additional positive contacts of the conditioning (CondPCs, $\chi^2_1 < 0.11$ $p > 0.74$, 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

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-pig relationship

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Key words:

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

Abstract:

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. Understanding and describing the quality of an HAR requires information on several aspects of the animal biology and emotional states (social, spatial and postural behaviours, physiological and cognitive states). Growing evidence shows that acoustic features of animal vocalisations may be indicators of emotional states. Here, we tested the hypothesis that the quality of vocal expression may indicate the quality of HAR. At weaning, 30 piglets were positively handled by an experimenter who talked to and physically interacted with them three times a day, while 30 other piglets only received the contact necessary for proper husbandry. After two weeks, we recorded the behaviours and vocalisations produced in the presence of the static experimenter for five minutes. We repeated this test two weeks later, after a conditioning period during which human presence with additional positive contact was used as a reward for all piglets. We hypothesized this conditioning period would lead to a positive human-piglet relationship for all piglets. As expected, piglets that were positively handled at weaning expressed a higher attraction toward the experimenter, and, after the conditioning, piglets that were not positively handled at weaning expressed a similar level of attraction than the positively handled ones. Piglets positively handled at weaning generally produced shorter grunts than the other ones. However the latter expressed more flexibility in call structure when vocalising close to a human, with a decrease of grunt duration and an increase in pitch, frequency range and noisiness in their grunt. This differential effect of proximity between groups of piglets was attenuated after the conditioning during a standard reunion with a static human but remained over time when the human was providing additional positive contacts. Results suggest that first, changes in vocal structure are consistent with indicators of positive states in the presence of a human. Second, increasing familiarity and proximity between a human and a pig may induce changes in the acoustic structure of its grunts. Third, a human providing additional positive contacts triggers more changes in vocalisation structure than by their presence only. We show that vocalisation structure may allow us to assess the quality of human-pig relationship.

Introduction

The process of domestication was conducted to shape physiology and morphology of domestic animal species, but also their behaviour. It notably has shaped interspecific interactions between human and non-human animals, by improving animals' capacity to use human signals to adapt their behaviour both decreasing fearfulness toward humans and increasing attention toward humans (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is important for animal welfare. Therefore, studying human-animal interactions and their consequences to understand the mechanisms of emergence and maintenance of a positive human-animal relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three major aspects: the ability of an animal to control its mental and physiological stability (Broom, 2011), the decrease of experiencing negatively perceived contexts and the increase in experiencing positively perceived contexts and species-specific behaviors (Peterson et al., 1995; Weerd & Day, 2009). A positive HAR is thought to be established through repeated positive interactions between the human and the non-human animal. Some of the mechanisms involved in this process are: accumulation of positive experiences through positive associative learning, modifications of cognitive biases, shaping expectations from the non-human animal toward the human. A positive HAR can be appreciated through behavioural and physiological measures, for example by assessing the expression of positive emotions [reviewed in (Rault et al. 2020)]. Several behavioural measures may help to define a positive HAR such as: short latency to approach and spatial proximity (Boivin et al., 2000; Schmied et al., 2008), body postures (Villain et al., 2020b) or play behaviour (Jerolmack, 2009). Contacts from a human such as stroking, may induce changes in body postures and exposition of body areas by the animal to the human, supposedly vulnerable [central neck area in cattle (Schmied et al. 2008), abdominal area in pigs (Rault et al., 2019)]. Such grooming solicitation may be markers of engagement, trust and motivation to interact with the human. In most cases, these behaviours are similar to those shown during intraspecific socio positive interactions, although there are some species specific behaviours [e.g., dog vs.. wolf (Gácsi et al., 2005)]. Vocal behaviour may also help defining the quality of an HAR. First, vocalisations are known to carry markers of the emotional states in several bird and mammal species (Briefer, 2012, 2020). Markers of emotional valence (positive versus negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Second, some vocalisations have been associated with positive interactions with humans, for example the cat – human communication : purring is thought to be derived from mother pup communication during nursing and is observed associated with care solicitation from humans; meowing, which is not observed during intra specific interactions is thought to emerge from associative learning during cat – human interactions (Brown & Bradshaw, 2014). This shows that HAR may elicit specific vocalisations from the non human animal toward the human.

In pigs, diversified evidence attest the possibility of a positive HAR. Animals may be handled by humans providing regular additional positive contacts, leading to the expression of a positive perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et al., 2015b). Pigs may recognise a human providing positive contacts compared to an unfamiliar one and adapt their behaviour accordingly (Brajon et al., 2015c). Pigs may be sensitive to human voice and respond accordingly (Bensoussan et al., 2019, 2020). Pigs vocalisations are diverse and linked to their emotional states, attested by the use of positive or negative call types (Briefer et al., 2019, 2022; Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related to the valence of a situation or the arousal a situation may trigger for the animal. For example, grunts, that are among of the most used vocal signals and various situations is now known to be a flexible call: shorter grunts have been associated with positive situations (Briefer et al., 2019, 2022; Friel et al., 2019), as well as higher formants (which are frequency peaks containing more energy than others) and a lower fundamental frequency during positive situations (Briefer et al., 2019, 2022). Grunt structure may also change according to the arousal of a situation, with a higher frequency range and a higher bandwidth when the arousal increases (Linhart et al., 2015). In order to determine if vocalisations may be used as non invasive indicators of the quality of human-pig relationship by themselves, we tested whether they could encode the quality of the human-pig relationship, through the vocal expression of emotional state. Because they are used in contexts of different valence and arousal and in most pigs, we studied the spectro-temporal structure of grunts. We predicted that if grunts carry information on the quality of the human-pig relationship, then 1. A period of positive handling given by a human should modulate vocal quality of piglets when in presence of the human, leading to grunts exhibiting markers of positive states (shorter grunts), 2. spatial proximity toward the human should influence the spectro-temporal structure of grunts (higher pitched grunts as the arousal increases).

Methods

Ethical note

The study was approved by the ethic committee CREEA and received the authorization no. APAFIS#17071-2018101016045373_V3 from the French Ministry of Higher Education, Research and Innovation. 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 piglets and its regulations.

Subjects and housing conditions

Sixty weaned female pigs (in two replicates from January to April 2019), *Sus scrofa domesticus*, bred from crosses between Large White and Landrace females and Piétrain males were used for this study from 28 to 62 days after birth. Animal housing and experiments took place at the experimental unit UE3P (UE 1421, INRAE France).

One piglet had to be excluded from our sample size to receive care/medication due to health issues independent from the experiment. From weaning at 28 days of age, piglets from the same litter and having similar weight (<1 kg difference) were housed by three in a 1.2 x 1.3m pen on plastic duckboard. Wooden panels were used to visually isolate pens. One metal chain per pen was used for enrichment. Food and water were available *ad libitum*. Artificial lights were turned on from 8:00 to 17:00 and temperature was maintained between 26 and 27 ºC. The experiment was carried out in two replicates and two identical rearing rooms were used (5 pens per room per replicate).

Treatment: positive handling et weaning

From day 28 (day of weaning) to day 39 of life, piglets were separated into two groups that experienced a different post-weaning period as follows:

- Non positively handled piglets (H piglets): Control piglets from 10 rearing pens, housed in the same room, received the minimal amount of daily contact with a stockperson (a 1.70m tall male who did the feeding, cleaning and health checkups). The stockperson wore a dark green shirt and pants and brown shoes.

- Positively handled piglets piglets (H+ piglets): Experimental piglets from the 10 other rearing pens, housed in another room, received the same daily care given by the same stockperson as for H piglets. They additionally received repeated sessions of additional human contacts. Each pen of three piglets received 29 sessions of 10 minutes, from day 28 (weaning) until day 39, occurring five days a week. Three sessions per day were performed (except on the day of weaning during which only two were done with a two-hour break in between). Each session took place in the rearing pen and the order of the interventions in the pens was balanced across days. The handling procedure, using gentle tactile contacts is described in supplementary material of Villain et al. (2020) and was similar to Tallet et al. (2014). Two experimenters performed these sessions (both women, both between 1.70-1.73 m tall, with a balanced number of pens attributed to each of them). The experimenters wore the same blue overalls and green boots each time they interacted with the piglets. The experimenters tried to imitate each others behaviours (remote video monitoring) to decrease variability.

This intense period of additional positive contacts for half of the piglets after weaning constituted the treatment of positive handling at weaning: positively handled piglets are referred to as H+ piglets and non positively handled piglets are referred to as H piglets to describe the early experimental treatment they experienced regarding a human, prior to the conditioning.

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

The conditioning took place between day 42 and 62 of age and lasted twelve days, with two trials per day and at least three hours between trials on the same day. Piglets were habituated to the test room for 10 minutes, by pen, two days before the start of the conditioning. All piglets (H and H+) were subjected to the same conditioning. The experimental design of the conditioning is already published in an article dedicated to the study of anticipatory behaviour (Villain et al., 2020).

Briefly, all piglets were individually trained to learn to associate two different stimuli with the arrival of two different (pseudo)-social partners: either two pen mates (partner = Conspecifics) or a familiar human (partner = Human). When entering the room, the piglets and the partner(s) would remain in the room for two minutes. Specifically, when the human was the partner, the human entered, sat on a bucket and positively interacted with the piglet for two minutes, in the same manner as additional contacts was provided to the H+ piglets during the previous period (see above section) (figure 1). Therefore, at the beginning of the conditioning phase, H+ piglets were already familiar with the human, whereas H piglets were unfamiliar with the human and only became familiar during the conditioning.

The same sessions occurred in both treatment groups (H and H+). It was thus excepted that, at the end of the conditioning, all piglets would be familiar with the human, but with a different degree in H+ and H- piglets, due to a different time of exposure (H+: period of positive handling at weaning + conditioning, H: conditioning only). 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). Indeed, first the two (pseudo) social contexts would have been difficult to compare (reunion between three piglets vs. reunion between one piglet and one human). Second, regarding the vocal behaviour, the caller among the group of three piglets would not have been identified reliably, making it difficult to study within individual vocal flexibility.

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 (see behavioural analyses section).

Standard Isolation/Reunion Tests

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

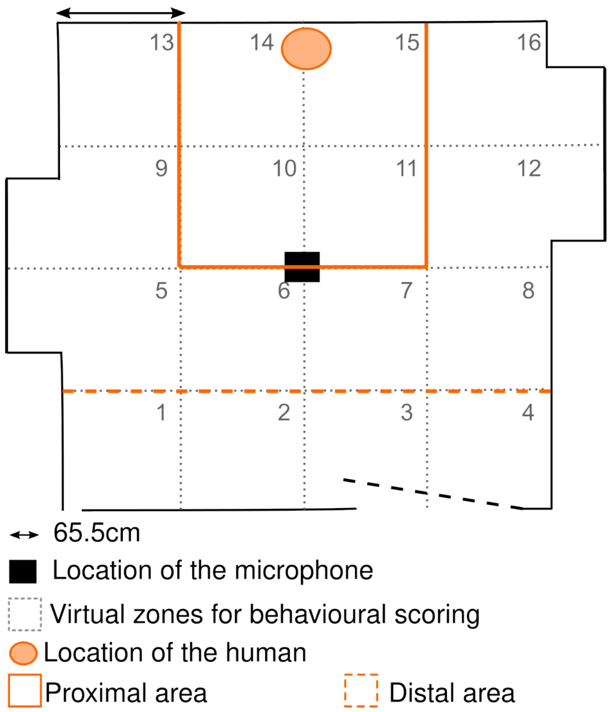

Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1, 2, 3, 4) were defined, suing the location of the human as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

Behavioural monitoring and analyses

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. The room was split into 16 virtual equally-dimensioned zones to assess the mobility and exploratory behaviour of the piglet. A proximal area, around the human was defined by merging four zones, a distal area was defined merging the four most distant zones from the human (figure 1).

The behaviours scored during the reunion of the Isolation/Reunion test and the sessions of additional positive contacts of the conditioning are available in table 1. Every time the shoulders of the piglet crossed a zone, a zone change was scored. Looks and watching behaviours were scored as point events, all other behaviours were scored as state events. Behavioural scores were then calculated to quantify global responses (see below).

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

Acoustic monitoring and analyses

Vocalisations were recorded with an AKG C314 microphone placed in the center of the room and one meter above the ground, connected to a Marantz MD661MK2 recorder. Vocalisations produced during each phase of the trial were manually annotated according to vocal type (grunt, squeal, bark, scream and mixed calls (Kiley, 1972)), after visual inspection of spectrograms using the 'Annotate' function of the Praat software (Boersma & Paul, 2001), version 6.0 from http://www.praat.org/. Checking the occurence of each call type in the several contexts of the study, we confirmed that 'grunt' was the call type used in all contexts and by most of the piglets in each context. So only the spectro-temporal structure of grunts was further analysed. For information, a table of the number of each call types recorded in each context as well as the number of individuals involved in the count is presented in the electronic supplementary material. We could not conduct a robust statistical analysis on call type utterance, due to the rarity (per subject and tests) of other vocalisations than grunt. (table S5).

A spectro-temporal analysis was performed with custom-written codes using the Seewave R package (Sueur et al., 2008) implemented in R (R Core Team, 2015). We first studied the spectral properties of the remaining background noise of the experimental room (electric noises and remaining low frequency noises from the rest of the building), using 20 examples of 0.5 second fragments. Since the first quartile (Q25) of the normalized spectrum of the background noise was 250Hz and the grunts are low frequency vocalisations, we decided to remove all frequencies below 200Hz in order to focus on the most relevant frequencies, using a 0.2-8 kHz bandpass filtering ('fir' function). As a consequence, all results presented in this study are on a 0.2-8kHz frequency range, and no conclusions on possible frequency components of grunts below this 200Hz threshold can be drawn here. To measure grunt duration, a 5% to maximal amplitude threshold was used ('timer' function). After normalisation, the following spectral parameters were calculated using the 'specprop' function

(FFT with Hamming window, window length = 512, overlap = 50%): mean (Q50), first (Q25) and third (Q75) quartiles, interquartile range (IQR), centroid and standard deviation (all in Hz). The grunt dominant frequency (in kHz) was also calculated (‘dfreq’, 50% overlapping FFTs, window length = 512), which is the mean over the grunt duration of the frequencies of highest energy of each window. Frequency peaks were detected and the minimal and maximal peaks were kept as descriptors (‘fpeaks’ function, window length = 512, peak detection threshold = 10% of the normalized amplitude). Measures of noisiness and entropy of the grunts were assessed using: Shannon entropy (sh), Spectral Flatness (Wiener entropy, sfm) and Entropy (H) [combining both Shannon and Temporal envelop entropy, length = 512, Hilbert envelop). Two vocal scores were used: the logarithm of grunt duration and a built-in spectral vocal score with all spectral parameters (see below). A table describing mean and range of variation of each acoustic parameter in the relevant contexts of the study is available in the supplementary material (table S4).

Statistical analyses

Behavioural and vocal response scores

The symmetrical distribution of all behavioural parameters on the one hand and all acoustic parameters on the other hand was visually inspected, and linear transformations were computed when necessary to reach symmetrical distribution (see tables 2, 3, 4). Two Principal Component Analyses (PCA, one for the behavioural analysis and one for the spectral acoustic analysis) were performed using all parameter having a symmetrical distribution (‘dudi.pca’ function from ‘ade4’ R package (Dray & Dufour, 2007) and ‘inertia.dudi’ function to extract the loadings). Indeed, PCAs are generally used to reduce the number of variables used in statistical models. It also generates quantifiable global descriptors of behaviours or acoustic parameters, since correlated parameters usually load on the same PC (McGregor, 1992). All PCs having an eigenvalue above one were kept and constituted response scores of behavioural (‘ReuPCs’ and ‘CondPCs’ in table 2 and 3 respectively) and vocal (‘VocPCs’, table 4) parameters. Only the duration of grunts was kept separated from the spectral parameters to keep it as a temporal parameter.

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).

	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**

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).

	CondPC1	**CondPC2**	**CondPC3**
Cumulative variance explained %	41	68.5	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
Latency to contact H	**-81.01**	-0.248	-2.83

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

	VocPC1	**VocPC2**	**VocPC3**

Cumulative variance explained %	59.769	76.807	87.712
Mean Dominant Frequency[1]	-13.558	**53.557**	2.220
Min frequency peak[1] (log)	-0.349	**58.758**	24.236
Max frequency peak[1]	**-43.023**	8.760	-9.537
Mode[2] (log)	-0.522	**66.248**	19.268
Mean[2] (log)	**-95.092**	-2.295	2.028
Q50[2] (log)	**-85.278**	0.280	-0.093
Q25[2] (log)	**-52.360**	19.327	0.985
Q75[2] (sqrt)	**-88.925**	-4.645	2.309
Centroid[2] (log)	**-95.092**	-2.295	2.028
Sd[2]	**-64.484**	-11.303	7.680
IQR[2]	**-87.981**	-5.851	2.640
Sfm[3] (sqrt)	**-94.344**	-3.189	0.962
Sh[3] (sqrt)	**-96.087**	-0.785	-0.175
H[3]	**-88.205**	-1.059	-1.063
Skewness[4]	28.032	-18.010	**48.652**
Kurtosis[4]	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

Statistical models

All statistics were carried out on R (R Core Team, 2015). Linear mixed effect models ('lmer' function, 'lme4' R package (Bates et al., 2014)) were built when studied variables were linear (behavioural and vocal scores, grunt duration) and one binomial generalized mixed effect model was built for binary parameters (occurrence of missed contacts initiated by human during the conditioning). The following subsections describe how models were built for each type of tests. In all models described below, the identity of the replicate ('1' or '2') was used as an interacting fixed factor, since the experiment was run in two identical replicates on two independent groups. The identity of the human ('AH' or 'AV') was used as interacting fixed factor in all models described below, since two experimenters were involved in the positive handling at weaning and in the session of additional positive contacts of the conditioning (but always the same human was attributed to a given piglet). The piglet was used as random factor to take into account the within-subject design.

Isolation/Reunion tests

The aim of this part was to test the effect of the positive handling at weaning treatment (H vs. H+ piglets) and additional human contacts during sessions of the conditioning on the piglet's reaction to human presence. Since the same Isolation/Reunion test was repeated before and after the conditioning, we used the variable 'Conditioning time' as a two level interacting factor ('before' or 'after' the conditioning) to test the effect of the conditioning.

```
Model1 <– lmer (Response variable ~ Treatment*Time + Treatment*Replicate + Treatment*HumanID + Time*Replicate + Time*HumanID + (1 | pigletID), data= dataBehaviourReunion).
```

Concerning the analysis of vocal behaviour, the isolation phase represents a negative social context for the piglets and may be used as a negative control when monitoring the effect of human presence on vocal expression of emotional states (Villain et al. 2020a). So, the two phases of the test were used to study the three way interaction between the treatment (H vs.. H+), the phase of the test (isolation vs.. reunion) and the time of the conditioning (before vs.. after). The following model was computed :

```
Model2 <– lmer (Vocal response score ~ Treatment*Phase*Time + Treatment*HumanID + Time*HumanID + Treatment*Replicate + Time*Replicate + (1 | pigletID/Time/Phase) , data= dataVocalIsolation + dataVocalReunion).
```

To go further, only the reunion phase was kept and a proximity variable was added. Indeed, the piglet could vocalise either when near the human or away from them and this spatial proximity was demonstrated as an important factor of changes of vocal features (Villain et al. 2020b). Thus, a two level proximity factor was built: either '1' when the piglet was in the proximal area (figure 1) or '0' when it was elsewhere in the room.

```
Model3 <- lmer (Vocal response score ~ Treatment*Time*InProxArea + Treatment*HumanID + InProxArea*HumanID + Treatment*Replicate + InProxArea*Replicate + Time*Replicate + Time*HumanID + (1 | pigletID/Time), data = dataVocalReunion).
```

Conditioning trials

The aim was to study the evolution of human-piglet relationship over the conditioning [the variable 'Trial number', used as a continuous variable], depending on the previous experience piglets had with the human [either already familiar (H+ group) or unfamiliar (H group) at the beginning of the conditioning]. Trial number (Trial) was also used as a random slope to take into account individual trajectories (Schielzeth and Forstmeier 2009). The following model was built to test the behavioural response scores (lmer) and the occurrence of missed contact initiated by the human during a session (presence/absence, binomial model, glmer):

```
Model4 <— (g)lmer (Behavioural Response score ~ Trial*Treatment + Trial*HumanID + Trial*Replicate + Treatment*Replicate + Treatment* umanID + (1+ Trial | pigletID), (family=Binomial), data= dataBehaviourConditioning).
```

For the analysis of vocal response scores, similarly to the Isolation/Reunion test, the piglet could vocalise either when near the human or away from them. We thus added the proximity factor in the analysis of vocal response variables. The following model was built :

```
Model5 <— lmer (Vocal Response score ~ Trial*Treatment*InProxArea+ Trial*HumanID + Trial*Replicate + Treatment*Replicate + Treatment*HumanID + HumanID*InProxArea + Replicate*InProxArea + (1+ Trial | pigletID), data= dataVocalConditioning).
```

Model validation and statistical tests

All linear models were validated by visual inspection of the symmetrical and normal distribution of the residuals. Anovas (‘car’ R package (Fox & Weisberg, 2011)) were computed on models to test for significant effects of explanatory variables. Following the Anova, when interactions were found significant, post hoc test were run on model interactions, correcting for multiple testing with Tukey contrasts (‘emmeans’ or ‘lstrends’ functions from ‘emmeans’ R package (Lenth, 2016), for categorical or continuous variables respectively). Results of the Anova, model estimates and pairwise post hoc comparisons are reported in the supplementary material (tables S1 and S2 for tests, table S3 for model estimates).

Results

Effect of the conditioning process on piglets' reaction to human presence (Isolation/Reunion tests)

Piglets that were not positively handled at weaning express a similar behavioural proximity to a human after a positive conditioning as positively handled ones.

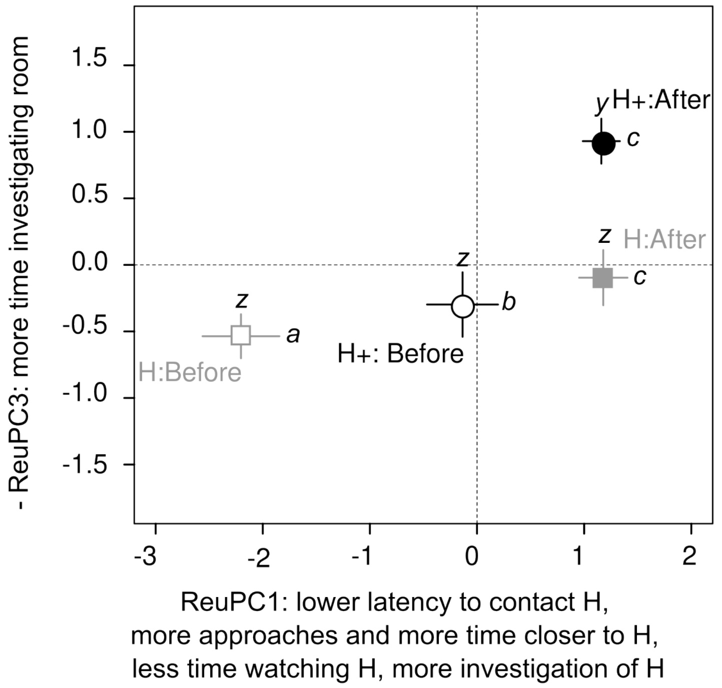

Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean ± SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey squares and H+ : black circles) and time (Before the conditioning: empty elements and After the conditioning: filled elements) on behavioural PC1 (letters a to c) and PC3 (letters z and y). Full statistical report is available as supplementary material (tables S1 S2 for statistical tests and S3 for model estimates)

The interaction between the treatment (positively handled piglets before the conditioning (H+) or not (H)) and the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3 ($\chi^2_1 = 28.0$, $p < 0.001$, and $\chi^2_1 = 3.7$, $p = 0.05$ respectively, figure 2) but not for ReuPC2 ($\chi^2_1 < 0.001$, p = 0. 99, supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was higher after the conditioning than before (H: after – before, t.ratio = 12.1, p <0.001 , H+: after – before t.ratio = 11.0, p < 0.001) and that before the conditioning, piglets that were positively handled at weaning had significantly higher ReuPC1 than non handled piglets (Before, H – H+: t.ratio = -2.1, p < 0.001), but not after (After, H – H+: t.ratio = 0.02, p = 1.0). According to the loadings, this means that piglets that were positively handled at weaning had a lower latency to contact the human, approached them more often and spent more time close to and investigating the human (ReuPC1) than non handled piglets, before the conditioning. This score increased after the conditioning and no evidence of a difference between treatments after the conditioning was found (figure 2). Post hoc tests on ReuPC3 showed a significant effect of the conditioning time only in piglets that were positively handled at weaning (H+: after – before, t.ratio = 5.2, p < 0.001, H: after – before, t.ratio = 2.6, p = 0.06). No difference in ReuPC3 was found between treatments before the conditioning (Before: H – H+, t.ratio = -0.75, p = 0.87), whereas positively handled piglets had a higher -ReuPC3 after the conditioning than before (After : H – H+, t.ratio = -3.2, p = 0.009). According to the loadings, this means that piglets that were positively handled at weaning expressed more investigation of the room after the conditioning than before. No evidence of any effect on ReuPC2 was found (table S2).

Positive handling at weaning decreases grunt duration even when no human is present with the piglet

Comparing the effect of the phase of the test (Isolation vs.. Reunion with the human), taking into account the conditioning time and the treatment, no evidence of any effect of neither the three way interaction ($\chi^2_1 < 0.62$, $p > 0.43$) nor two way interactions of interest was found (treatment: phase, conditioning time:phase, conditioning time: treatment interactions : χ^2_1 <3.5, $p > 0.06$, table S2) in any of the scores. However, grunts produced by positively handled piglets were shorter than grunts produced by non handled piglets ($\chi^2_1 = 5.5$, $p = 0.02$, estimates of log(duration)[95% confidence interval]: -1.25[-1.32;-1.19] and -1.12[-1.2;-1.1] respectively in H+ and H piglets, table S3). Single effects of the phase of the test were significant for grunt duration and all AcPCs ($\chi^2_1 > 6.6$, $p < 0.01$, table S1). Grunts produced during the reunion phase with the human were shorter (estimates of log(duration) : -1.32[-1.37;-1.26] vs.. -1.06[-1.12;-1.00]) and, according to the loadings, grunts produced during the reunion phase had a higher frequency range, higher bandwidth and a higher noise component (-VocPC1: 0.78[0.48;1.08] vs.. 0.34[0.03;0.66]), higher pitched (VocPC2: -0.18[-0.36;0.01] vs.. -0.46[-0.65;-0.28]) and their spectrum had a higher skewness and kurtosis (VocPC3: -0.25[-0.37;-0.14] vs.. -0.11[-0.23;0.01]), compared to the isolation phase.

The conditioning process attenuates the effect of proximity on grunts vocal features in non handled piglets

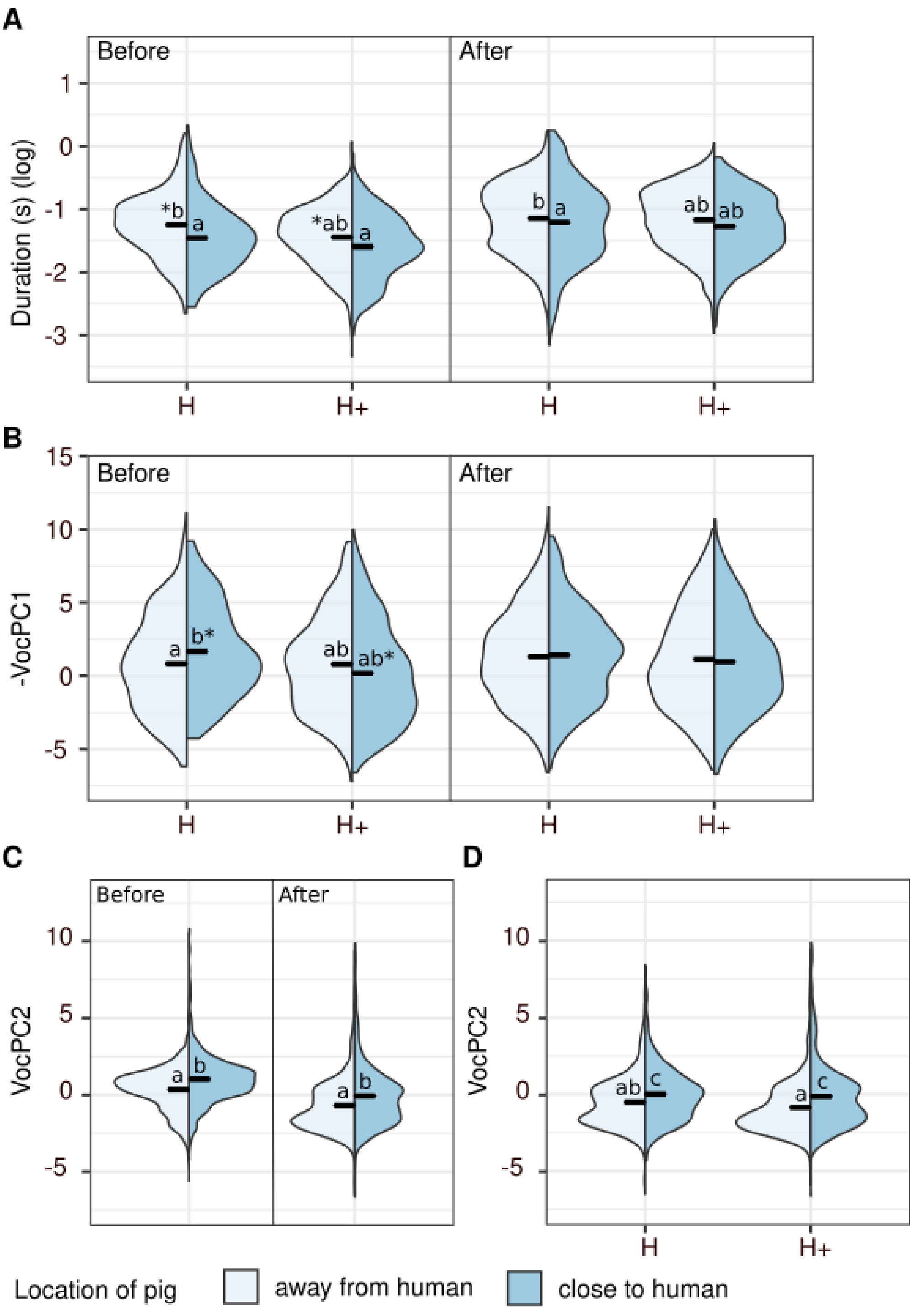

Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect of conditioning (before or after), treatment (H or H+), and location of the piglet relatively to the human (close: dark blue or away from them: light blue). Violin plots representing 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 (C) 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).

During the five-minute reunion, the piglet was scored either as close to the human or away from them. The three way interaction of the conditioning time, the treatment and the location was significant for grunt duration, -VocPC1 and VocPC3 ($\chi^2_1 > 4.9$, $p < 0.03$). Post hoc tests revealed that grunts produced closer to the human were shorter than the ones produced further away, but only in piglets that were not positively handled at weaning, effect being stronger before the conditioning than after it (H piglets: away – close, z.ratio = 6.3, p < 0.001 before and z.ratio = 4.1 p < 0.001 after the conditioning; H+ piglets: away – close z.ratio < 1.98 p > 0.19, figure 3A). -VocPC1 was higher, i.e. grunts had a higher frequency range, bandwidth and were noisier when produced closer to the human than further away, but only in non handled piglets and before the conditioning (H piglets: away – close, z.ratio = -3.34, p = 0.005 before and z.ratio = -1.23 p = 0.61 after the conditioning; H+ piglets: away – close, z.ratio < 0.36 p > 0.21, figure 3B). For VocPC2, the three way interaction did not reach significance ($\chi^2_1 = 3.3$, $p = 0.07$), 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 ($\chi^2_1 = 10.3$, $p = 0.001$) on the one hand, and between the location and the treatment ($\chi^2_1 = 4.2$, $p = 0.04$) 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). The last two-way interaction of interest between the conditioning time and the treatment did not reach significant level ($\chi^2_1 = 0.80$, $p = 0.37$). For VocPC3, post hoc tests did not reach significant levels (|z.ratio| < 2.3 p > 0.09 for any comparison) .

Emergence of positive perception of human (effect of additional positive contacts sessions over the conditioning)

The conditioning process increases behavioural proximity

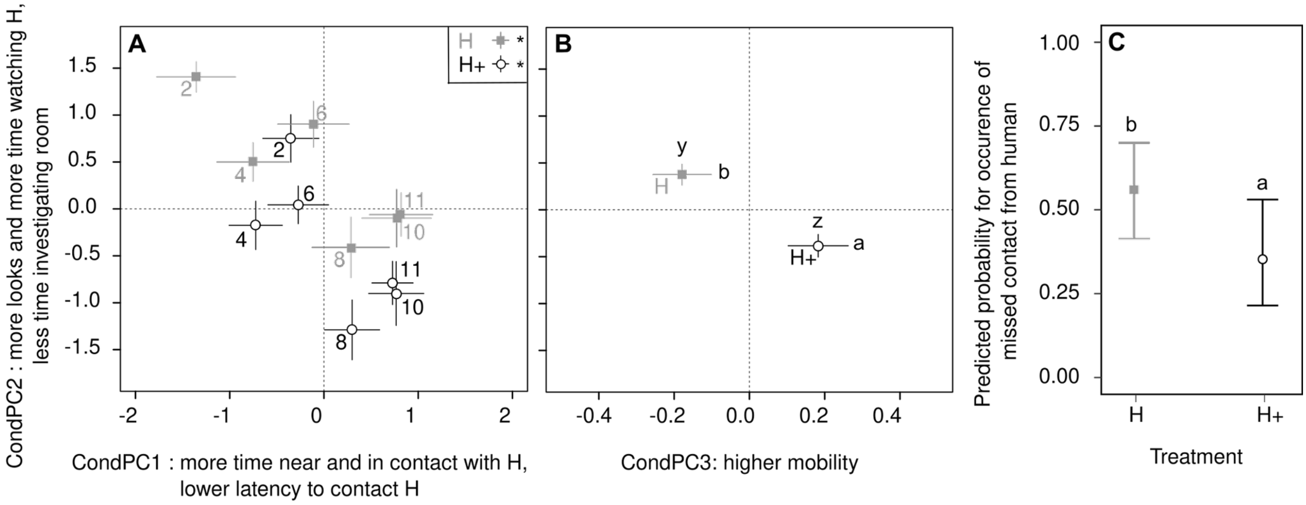

Figure 4: Effect of trial number over the conditioning on spatial behaviour and proximity to the human during the 2min sessions of additional positive contacts of the conditioning. (A, B) Mean ± SE per group. (A) Single effect of trial number on behavioural scores CondPC1 and condPC2 according to treatment (H: grey, H+: black). (B) Single effect of treatment on behavioural score CondPC3 and CondPC2. (C) Single effect of treatment on predicted probability of occurrence of at least one missed contact by the human, mean estimates ± 95% confidence interval from the generalized mixed effect model. Stars in the legend box represent significant effect of the trial number (A), different letters represent significantly different groups (B and C). Full statistical report is available as supplementary material (tables S1 et S2 for statistical tests, table S3 for model estimates).

No evidence of any effect of the interaction between the treatment [positively handled piglets before the conditioning (H+) or not (H)] and the trial number was found for all behavioural scores (CondPC1, CondPC2 and CondPC3, table 3). Independently from the treatment, the higher the trial number the higher CondPC1 ($\chi^2_1 = 59.3$, $p < 0.001$, slope estimate [95% confidence interval]: 0.20 [0.15 : 0.25]) and the lower CondPC2 was ($\chi^2_1 = 48.6$, $p < 0.001$, slope estimate: -0.17 [-0.22 : -0.12]). According to the loadings, over the conditioning, piglets decreased the latency to contact the human, made more contacts, spent more time in the proximal area and in contact with the human (condPC1), decreased the number of looks to the human, spent less watching the human and more time investigating the room (CondPC2) (figure 4A). Independently from the trial number, positively handled piglets had a lower CondPC2 and a higher CondPC3 than the non handled ones ($\chi^2_1 = 12.8$, $p < 0.001$ and $\chi^2_1 = 7.0$, $p = 0.008$ respectively), meaning that piglets that were positively handled at weaning expressed a fewer number of looks to the human, spent less time watching them and more time investigating the room (CondPC2) and crossed more virtual zone during the test (CondPC3) (figure 4B). The probability of having at least one missed contact by the human during a session was lower for positively handled piglets than non handled ones ($\chi^2_1 = 9.57$, $p = 0.002$, figure 4C), with no interaction with the trial number ($\chi^2_1 = 0.22$, $p = 0.064$).

Grunt acoustic features depends on spatial proximity to human

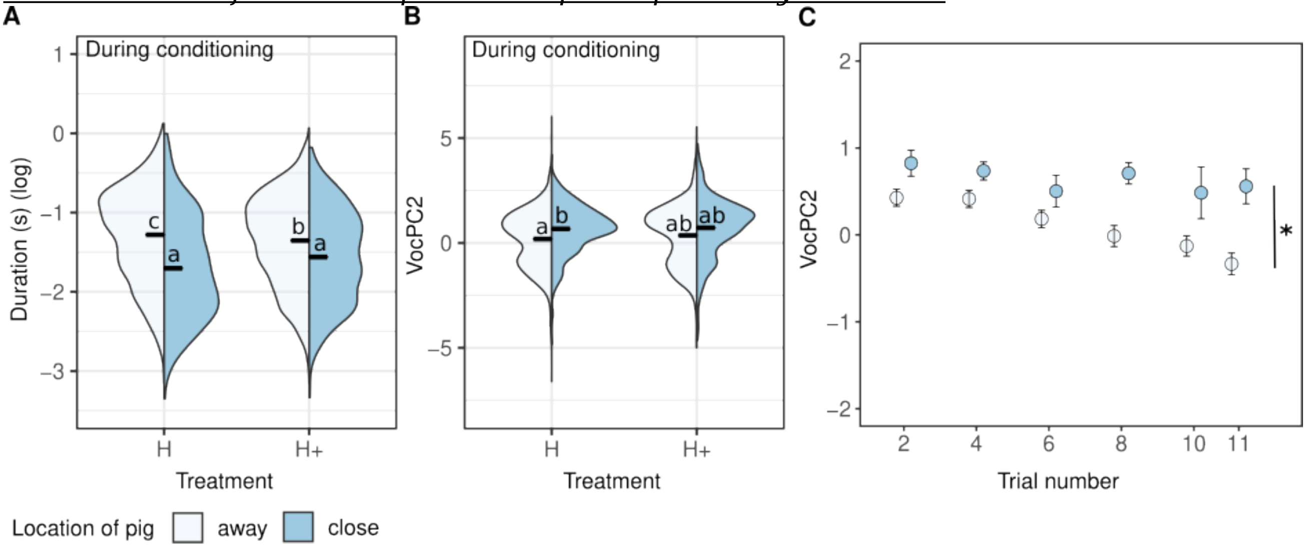

Figure 5: Evolution of vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human '(close': dark blue) or elsewhere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

During the sessions of additional positive contacts of the conditioning, the three-way interaction between the trial number, the treatment and the location was not significant for any of the vocal scores ($\chi^2_1 < 0.18$, $p > 0.67$), allowing the analysis of the two way interactions of interest. The interaction between treatment and the trial number was not significant for all vocal scores ($\chi^2_1 < 2.5$ p > 0.11), however grunt duration decreased over the conditioning sessions (trial number:replicate interaction, $\chi^2_1 < 5.3$ p = 0.02, slope estimate -0.03[-0.04;-0.01] for the lower slope, table S1 and S3). Independently from the trial number, grunt duration was lower when piglets were located close to the human and this effect was stronger in non handled piglets than positively handled piglets (treatment:location interaction: $\chi^2_1 = 15.8$ p < 0.001, away vs.. close, H piglets: z.ratio = 10.2 p < 0.001, H+ piglets: z.ratio = 6.86 p < 0.001, figure 5A). VocPC2 was higher when piglets were close to the human, but only in non handled piglets (treatment:location interaction, $\chi^2_1 = 7.6$ p = 0.005, pairwise comparisons away vs. close, in H: z.ratio = -4.9 p z 0.001 and in H+: z.ratio = -2.0 p = 0.21), meaning that non handled piglets produced higher pitched grunts when closer to the human (figure 5B). The effect of the location on -VocPC1 and VocPC2 depended on the trial number (trial number : location interaction, $\chi^2_1 = 3.97$ p = 0.05 and $\chi^2_1 = 6.1$ p = 0.01 respectively): -VocPC1 and VocPC2 were higher when closer to the human with a greater extent later in the conditioning than earlier (slope comparison away – close, -VocPC1 : z.ratio = -1.80 p = 0.07, VocPC2 : z.ratio = -2.34 p = 0.02). According to the loadings, this means that the frequency range, bandwidth and noisiness of grunts (-VocPC1) as well as the pitch (VocPC2) decreased over the conditioning when piglets were located away from the human but remained high when piglets were close (figure 5C and 5D).

Impact of human identity on piglets behaviour and grunt structure

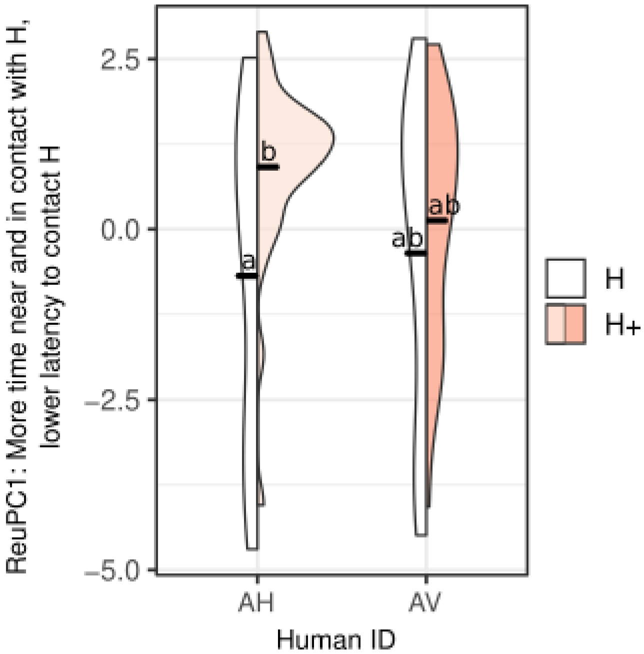

Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Different letters represent significantly different groups. Full statistical report is available as supplementary material (tables S1 and S2 for statistical tests, table S3 for model estimates).

Since half of the piglets had been assigned to one human experimenter and the other half to another one, the identity of the human was included in the model. This allowed to test interactions between the identity of the human and the treatment of positive handling at weaning on the one hand and the conditioning time on the other hand.

During the reunions of the Isolation/Reunion test, the interaction between treatment and human identity was significant for the first behavioural proximity score (ReuPC1, $\chi^2_1 = 6.01$, p = 0.01) but not the others (ReuPC2 and ReuPC3 ($\chi^2_1 < 1.98$, p > 0.16, table S1). The effect of treatment on ReuPC1 was higher when piglets were handled by the human ‘AH’ (H vs. H+, AH: t.ratio = -4.77, p < 0.001, figure 6). When the human ‘AV’ handled the piglets, for which ReuPC1 scores exhibited intermediate values, treatment was not significant (AV, H vs. H+: t.ratio = -1.33, p = 0.56). These interacting effects of the human identity and treatment on behaviour were not found when considering the reunions of the conditioning ($\chi^2_1 < 1.32$, $p > 0.25$ for all CondPCs, table S1).

Interactions between the human identity and conditioning time were not significant, neither considering the reunions of the Isolation/Reunion test (ReuPCs, $\chi^2_1 < 0.642$, $p > 0.42$, tables S1), neither the trial number during the session of additional positive contacts of the conditioning (CondPCs, $\chi^2_1 < 0.11$ $p > 0.74$, table S1).

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

Vocal response score	Fixed effect	Levels	Estimate	Lower.95% CI	Upper.95% CI	Statistic	P-value
Reunion of the Isolation/Reunion test							
VocPC2	humanID	AH	0.154	-0.119	0.427	$\chi^2_1 = 4.94$	P = 0.03
		AV	-0.292	-0.571	-0.012		
Sessions of additional positive contacts of the conditioning							
-VocPC1	humanID	AH	0.091	0.037	0.144	$\chi^2_1 = 4.69$	P = 0.03
		AV	0.076	0.021	0.132		
VocPC2	humanID*InProxArea	AH – away	0.317	0.110	0.524	Z-ratio = -1.23	P = 0.60
		AH – close	0.402	0.161	0.643		
		AV – away	0.027	-0.182	0.236	Z-ratio = -5.77	P < 0.001
		AV – close	0.462	0.212	0.712		
VocPC3	humanID * Trial number	AH – Trial number	-0.048	-0.070	-0.026		
		AV – Trial number	-0.007	-0.031	0.016	Z-ratio = -2.82	P = 0.005

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

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 (χ^2_1 = 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, VocPC3 changed differently when piglets were handled by the human 'AH' or 'AV', as showed by the significant interaction between trial number and human identity (χ^2_1 = 8.0, p = 0.005): the skewness and kurtosis of grunts decreased over the conditioning when 'AH' was interacting with the piglets, but not 'AV' (see slope estimates, table 5). No evidence of any effect of human identity was found on grunt duration neither during the Isolation/Reunion tests nor during the sessions of additional positive contacts of the conditioning (table S1).

Discussion

Behavioural evidence of a rapid establishment of interest and proximity toward a human providing additional positive contacts

The standard reunion test with the human before the conditioning showed that the treatment of positive handling at weaning succeeded in creating two different levels of human-piglet relationship (H and H+), as positively handled piglets expressed a higher attraction toward the human than non handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al., 2020). 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. 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). So it seems that the conditioning process allowed non handled piglets to compensate the lack of positive handling before the conditioning and develop a similar proximity toward the human. Two minute daily sessions of additional positive contacts changed positively the perception of the human for the piglets, and thus their willingness to interact with them. Since no evidence of any interaction between time and treatment was found, no conclusion on differential developmental trajectories between treatments can be drawn, but a parallel development of the human-piglet relationship in both groups, when considering the proximity.

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 & 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 & 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 et al., 2009).

Overall, the behavioural monitoring showed that two minute sessions of positive additional contacts per day are sufficient to increase proximity to a human to similar levels as when piglets were previously familiarised for 2 weeks, even when piglets experienced social isolation. But it did not allow the non handled piglets to express natural exploratory behaviours as the positively handled piglets. 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.

Links between vocal expression and positive HAR

In this study, piglets were subjected to two types of interactions with the human: one in a standard reunion test during which, no movements nor speech was produced by the human. The second type of reunion consisted of sessions of additional positive contacts, during which the human actively interacted with the piglet, providing contacts and producing speech. These two types of interactions had different effects on vocal expression, which allows us to evaluate the origin and functions of the vocal flexibility expressed in grunt structure. 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.

Human mere presence affects vocal expression according to previous experience

Social isolation was associated with longer and lower pitched grunts with a downshifted frequency spectrum. A reunion with a static human changed grunt structure to shorter, higher pitched with an upshifted frequency spectrum and this was observed independently from the treatment (H or H+) and the conditioning time (before or after). In terms of emotional indicators, similar changes in acoustic features of grunts were already found in studies focusing on vocal markers of valence in pigs (Briefer et al., 2019, 2022; Friel et al., 2019; Villain et al., 2020) and are also in line with previous results in similar contexts in relation to the human presence (Villain et al., 2020b). These results, combined with the behavioural results, may validate The symmetrical distribution of some vocal parameters encoding of positive emotions (shorter and higher pitched grunts) in the context of a reunion with a human, potentially releasing the piglets from the stress of isolation.

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

We showed that the proximity to the human changed the structure of piglets grunts and that proximity significantly interacted with the treatment and the conditioning time. Indeed, similarly to a previous study (Villain et al. 2020a, 2020b), during the standard reunion test (no contact from the human), piglets produced shorter and higher pitched grunts with an upshifted frequency spectrum when closer to the human. It was especially the case in non handled piglets, before the conditioning. These results may be linked to the reactivity to the human, non handled piglets being more reactive to the presence of a human than positively handled piglets. Indeed, before the sessions of the conditioning, positively handled piglets were habituated to a human interacting positively when present whereas non handled piglets were not, hence, during the first standard reunion test, when the human is present but do not interact with the piglet, positively handled and non handled piglets may have diverging expectations regarding the presence of the static and silent human. As positively handled piglets received positive contacts every time they were in the presence of the human, they may have expected positive contacts when approaching and experienced an absence of reward during the test. This has already been hypothesised in piglets deprived from human voice during interactions after a period of habituation to it (Bensoussan et al. 2020). On the contrary, piglets that were not positively handled at weaning never experienced additional positive contacts and being close to a human, having the possibility to investigate them may be some kind of reward after the period of total isolation. After the conditioning, piglets from both treatments were conditioned to receive additional positive contacts and both groups had experienced a first standard reunion test, so they may both experience an absence of reward during the test, which may explain a lower reaction to proximity to the human, and thus fewer changes on grunt spectro-temporal features.

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

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 natural foraging 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 us to address these hypotheses.

Providing rewarding additional positive contacts changes the structure of grunts

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

Effect of human identity on piglets' perception: perspectives on HAR

We found that the identity of the human had effects on behavioural and vocal response scores. Piglets that were handled by the human 'AH' had higher values of behavioural proximity (ReuPC1) than piglets handled by the human 'AV' during reunion test after a period of isolation. This effect was not found during conditioning sessions. 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. Additionally, when the human 'AH' was in the room, piglets produced grunts with a more upshifted frequency spectrum and a higher pitch than when the human 'AV' was in the room. If upshifted grunts may be a indicator of positive higher arousal, then we may conclude that 'AH' was more likely to trigger higher positive states than 'AV'. Interestingly, the human identity and the spatial proximity had different effects on piglets grunts during sessions of additional positive contacts but not when the human was static during the standard reunion test. Hence, it is possible that the way one human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering positive emotions and thus modifications of grunt structure. Several evidence exists in the literature that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al., 2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We may question the efficiency of different human features to generate a positive HAR. In our study, both humans that interacted with the piglets wear exactly the same clothes and standardized their tactile interactions toward the piglets 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. It thus remains unclear whether experimenters interacted differently or if they were initially perceived differently by piglets. 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). 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.

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

Authors contributions

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

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Data availability

The datasets used for the study are available at (Villain et al., 2022). The folder contains all datasets and a readme to match the type of analysis to the proper dataset. 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.

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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	2	3.291	0.287	11.466	<0.001
Treatment * HumanID					
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+	0.812	0.271 -		3.003	0.003
Trial number | Batch					
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+	before		-0.134	0.220	-0.571	0.302
Conditioning time | Batch						
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	2		-2.437	0.222	-2.878	-1.996
Treatment * HumanID						
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+	before		1.583	0.055	1.474	1.692
Treatment | Batch						
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+	2	1.695	0.060	1.575	1.814
Conditioning time | Batch					
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		-0.482	0.202	-0.877	-0.087
Conditioning time					
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+	1	before	-1.571	0.054	-1.677	-1.465
Conditioning time | Batch						
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+	1	before	0.434	0.322	-0.198	1.066
Conditioning time | Batch						
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+	1	before	0.663	0.164	0.342	0.984
In prox. area | Conditioning time						
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	before		1.096	0.274	0.558	1.634
Treatment * In prox. area						
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+		1	-0.044	0.139	-0.316	0.229
In prox. area | Batch						
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	2		0.146	0.162	-0.171	0.463
Conditioning time | Batch						
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+	1	before	-0.427	0.105	-0.634	-0.221
Conditioning time | Batch						
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		0.002	0.106	-0.210	0.214
Occurence of missed contact from human					
Treatment					
H	-	-0.110	0.188	-0.479	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+	1	-1.567	0.044	-1.654	-1.481
Trial number | Batch					
1		-0.053	0.009	-0.070	-0.036
2		-0.025	0.009	-0.042	-0.008
Treatment | Batch					
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		0.109	0.031	0.048	0.169
Treatment | Batch					
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+	2	-2.033	0.218	-2.460	-1.606
In prox. area | Batch					
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+	1	0.340	0.119	0.108	0.572
Trial number * In prox. area					
0		-0.091	0.010	-0.110	-0.072
1		-0.054	0.017	-0.088	-0.021
Trial number | Batch					
1		-0.045	0.015	-0.074	-0.015
2		-0.100	0.015	-0.130	-0.071
In prox. area * HumanID					
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)**				
Conditioning time	**Treatment**	**Phase**	**Ncalls**		**Conditioning time**	**Treatment**	**In prox. area**	**Ncalls**		**Time in conditioning**	**Treatment**	**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 of parameter	**Sd of parameter**				**Mean of parameter**	**Sd of parameter**				**Mean of parameter**	**Sd of parameter**
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

		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)														
after	H	iso	1817.653	385.617	after	H	0	1885.087	426.846	early	H	0	1494.859	294.318
		reuH	1879.520	423.215			1	1868.693	410.882			1	1628.225	304.585
	H+	iso	1842.219	428.577		H+	0	1887.656	457.439		H+	0	1443.195	263.108
		reuH	1878.859	453.032			1	1837.232	442.185			1	1472.662	285.439
before	H	iso	1769.750	442.237	before	H	0	1811.102	443.617	late	H	0	1524.356	294.879
		reuH	1822.634	433.496			1	1851.117	414.251			1	1581.281	341.346
	H+	iso	1687.113	390.208		H+	0	1812.031	445.991		H+	0	1507.883	269.191
		reuH	1786.125	457.160			1	1736.176	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	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)														
after	H	iso	2724.508	901.531	after	H	0	2855.251	962.026	early	H	0	1907.359	821.008
		reuH	2838.172	964.096			1	2800.454	966.843			1	2271.884	873.368
	H+	iso	2797.501	989.550		H+	0	2871.378	1036.874		H+	0	1795.251	756.385
		reuH	2841.678	1032.082			1	2731.682	1032.696			1	1886.704	797.162
before	H	iso	2667.213	1043.411	before	H	0	2718.417	1054.461	late	H	0	2005.637	806.336
		reuH	2716.523	1026.942			1	2724.730	979.535			1	2132.724	873.945
	H+	iso	2483.126	934.606		H+	0	2770.862	1082.494		H+	0	2019.569	718.732
		reuH	2669.632	1139.513			1	2509.778	1182.463			1	2167.271	749.616
Centroid (Hz)														
after	H	iso	1817.653	385.617	after	H	0	1885.087	426.846	early	H	0	1494.859	294.318
		reuH	1879.520	423.215			1	1868.693	410.882			1	1628.225	304.585
	H+	iso	1842.219	428.577		H+	0	1887.656	457.439		H+	0	1443.195	263.108
		reuH	1878.859	453.032			1	1837.232	442.185			1	1472.662	285.439
before	H	iso	1769.750	442.237	before	H	0	1811.102	443.617	late	H	0	1524.356	294.879
		reuH	1822.634	433.496			1	1851.117	414.251			1	1581.281	341.346
	H+	iso	1687.113	390.208		H+	0	1812.031	445.991		H+	0	1507.883	269.191
		reuH	1786.125	457.160			1	1736.176	462.373			1	1563.601	289.547
Sd (Hz)														
after	H	iso	2134.495	176.184	after	H	0	2145.795	174.256	early	H	0	1990.877	174.232
		reuH	2144.085	175.255			1	2141.372	177.553			1	2047.922	175.263
	H+	iso	2153.259	199.831		H+	0	2147.761	186.968		H+	0	1942.072	169.828
		reuH	2140.791	187.566			1	2120.019	192.350			1	1940.150	184.449
before	H	iso	2069.712	201.094	before	H	0	2111.660	193.862	late	H	0	2007.860	175.699
		reuH	2096.807	194.727			1	2070.054	195.140			1	1992.677	170.148
	H+	iso	2022.945	173.442		H+	0	2106.114	205.720		H+	0	1974.469	164.606
		reuH	2095.007	211.699			1	2071.601	217.408			1	1964.886	163.211
IQR (Hz)														
after	H	iso	2421.766	863.329	after	H	0	2524.720	902.648	early	H	0	1619.915	797.974
		reuH	2507.599	906.090			1	2468.73	912.873			1	1961.994	849.624

								1						
	H+	iso	2492.962	947.609		H+	0	2546.763	981.693		H+	0	1505.074	739.851
		reuH	2513.318	978.741			1	2398.638	986.570			1	1585.150	779.768
before	H	iso	2350.176	993.024	before	H	0	2389.927	1001.070	late	H	0	1721.745	785.012
		reuH	2370.665	976.677			1	2344.219	935.061			1	1812.245	828.439
	H+	iso	2158.716	893.869		H+	0	2437.013	1036.722		H+	0	1738.078	702.471
		reuH	2334.358	1094.079			1	2175.986	1135.706			1	1859.715	720.425
Sfm														
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