1 The use of pigs vocalisation structure to assess the quality of
2 human-pig relationship

3 Authors

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12 Key words

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

15 Abstract

16   Studying human-animal interactions in domestic species and how they affect the establishment of a
17   positive Human-Animal Relationship (HAR) may help us improve animal welfare and better
18   understand the evolution of interspecific interactions associated with the domestication process.
19   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 vocal structure 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 contacts 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 produced shorter grunts than the other ones, regardless of the context of recording, which may indicate a more positive affect. During reunions with the static experimenter, a more positive HAR was associated with a decrease in vocal reactivity to human proximity. However, during reunions with the experimenter providing additional positive contacts and over the conditioning, proximity to the human systematically triggered shorter and higher pitched grunts, indicator of positive a emotional state. Results first show that changes in vocal structure are consistent with indicators of positive states in the presence of a human. Second, these changes are stronger when the human positively interact with the piglets, supposedly emphasizing a higher positive arousal state during these interactions. We show that vocalisation structure may be a promising indicator of 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,

53   2011), the decrease of experiencing negatively perceived contexts and the increase in experiencing

54   positively perceived contexts and species-specific behaviors (Peterson et al., 1995; Weerd & Day,

55   2009). A positive HAR is thought to be established through repeated positive interactions between

56   the human and the non-human animal. Some of the mechanisms involved in this process are:

57   accumulation of positive experiences through positive associative learning, modifications of

58   cognitive biases, shaping expectations from the non-human animal toward the human. A positive

59   HAR can be appreciated through behavioural and physiological measures, for example by assessing

60   the expression of positive emotions [reviewed in (Rault et al. 2020)]. Several behavioural measures

61   may help to define a positive HAR such as: short latency to approach and spatial proximity (Boivin

62   et al., 2000; Schmied et al., 2008), body postures (Villain, Lanthony, et al., 2020) or play behaviour

63   (Jerolmack, 2009). Contacts from a human such as stroking, may induce changes in body postures

64   and exposition of body areas by the animal to the human, supposedly vulnerable [central neck area in

65   cattle (Schmied et al. 2008), abdominal area in pigs (Rault et al., 2019)]. Such grooming solicitation

66   may be markers of engagement, trust and motivation to interact with the human. In most cases, these

67   behaviours are similar to those shown during intraspecific socio positive interactions, although there

68   are some species specific behaviours [e.g., dog vs. wolf (Gácsi et al., 2005)]. Vocal behaviour may

69   also help defining the quality of an HAR. First, some vocalisations type have been associated with

70   positive interactions with humans, for example the cat – human communication : purring is thought

71   to be derived from mother pup communication during nursing and is observed associated with care

72   solicitation from humans; meowing, which is not observed during intra specific interactions is

73   thought to emerge from associative learning during cat – human interactions (Brown & Bradshaw,

74   2014). This shows that HAR may elicit specific vocalisations from the non human animal toward the

75   human. Second, vocalisation structure is known to carry markers of the emotional states in several

76   bird and mammal species (Briefer, 2012, 2020) and markers of emotional valence (positive versus

77   negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Since

78   positive or negative HAR is likely to affect the emotional state of animals, it is likely that it may be

79   reflected in the structure of the produced vocalisations.

80   In pigs, diversified evidence attest the possibility of a positive HAR. Animals may be handled by

81   humans providing regular additional positive contacts, leading to the expression of a positive

82   perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests

83   showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et

84   al., 2015b). Pigs may recognise a human providing positive contacts compared to an unfamiliar one

85   and adapt their behaviour accordingly (Brajon et al., 2015c). Pigs may be sensitive to human voice

86   and respond accordingly (Bensoussan et al., 2019, 2020). Pigs vocalisations are diverse and linked to

87   their emotional states, attested by the use of positive or negative call types (Briefer et al., 2019, 2022;

88   Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related
89   to the valence or the arousal a situation may trigger for the animal. For example, the grunt, a contact
90   call, is used in various contexts and is now known to be a flexible call. Positive situations have been
91   associated with shorter grunts compared to negative ones (Briefer et al., 2019, 2022; Friel et al.,
92   2019), as well as higher formants (which are frequency peaks containing more energy than others)
93   and a lower fundamental frequency during positive situations (Briefer et al., 2019, 2022). Grunt
94   structure may also change according to the arousal of a negative situation: the higher the arousal in
95   the negative state the higher the frequency range and bandwidth (Linhart et al., 2015) and the longer
96   (Puppe et al., 2005) the grunts. Variation in grunt spectro-temporal structure in positive situations of
97   different arousal is still unknown.

98   In order to determine to what extent vocalisations structure could be used as non invasive indicator
99   of the quality of human-pig relationship, we tested whether varying the degree of familiarity and the
100 quality of the human-pig interactions could modulate the spectro-temporal structure of vocalisation,
101 through the vocal expression of emotional state. Because it was suggested to study vocal markers of
102 emotions within the same call type (Briefer, 2020) and because grunts are the most commonly
103 produced call in various contexts, we studied the spectro-temporal structure of grunts. We predicted
104 that if grunts reflect the quality of the human-pig relationship, then 1. A period of positive handling
105 given by a human should modulate piglets vocal expression in presence of the human, leading to
106 grunts exhibiting markers of positive states (higher pitched and shorter grunts), 2. Spatial proximity
107 toward the human should influence the spectro-temporal structure of grunts (higher pitched and
108 shorter grunts).

Methods

Ethical note

111 The study was approved by the ethic committee CREEA and received the authorization no.
112 APAFIS#17071-2018101016045373_V3 from the French Ministry of Higher Education, Research
113 and Innovation. UE3P, where the experiment was carried out, is an experimental unit authorized by
114 the French Ministry of Agriculture to breed animals for experimentation under the number D35-275-
115 32. This authorization includes a derogation to follow the directive 2008/120/EC relative to the
116 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 at 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.

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

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

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

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

168   The same sessions occurred in both treatment groups (H and H+). It was thus excepted that, at the
169   end of the conditioning, all piglets would be familiar with the human, but with a different degree in
170   H+ and H- piglets, due to a different time of exposure (H+: period of positive handling at weaning +
171   conditioning, H: conditioning only). Sessions of reunions with social partners were not studied here
172   because they were part of an analysis on vocal expression of positive anticipation reported earlier
173   (Villain, Hazard, et al., 2020).

174   For every second trial, the two-minute reunions with the human were analysed by the same person:
175   trials number 2, 4, 6, 8, 10 and 11 (see behavioural analyses section).

Standard Isolation/Reunion Tests with a static and silent human

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

↔ 65.5cm
■ Location of the microphone
⬚ Virtual zones for behavioural scoring
🟠 Location of the human
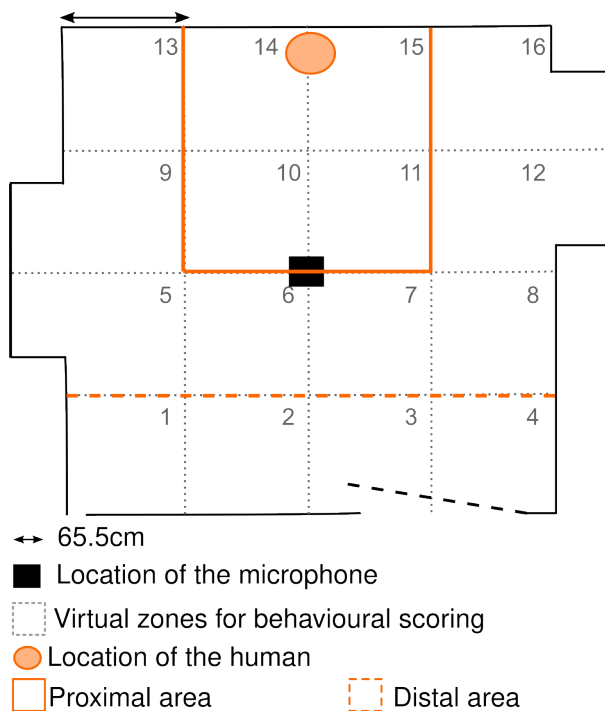▭ Proximal area   ⬚ Distal area

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

183   Sessions and tests were recorded using a camera (Bosh, Box 960H-CDD) and behaviours were
184   scored *a posteriori* on videos using *The Observer XT 14.0* (Noldus, The Netherlands) software. The
185   room was split into 16 virtual equally-dimensioned zones to assess the mobility and exploratory
186   behaviour of the piglet. A proximal area, around the human was defined by merging four zones, a
187   distal area was defined merging the four most distant zones from the human (figure 1).

188   The behaviours scored during the reunion of the Isolation/Reunion test and the sessions of
189   additional positive contacts of the conditioning are available in table 1. Every time the shoulders of
190   the piglet crossed a zone, a zone change was scored. Looks and watching behaviours were scored as
191   point events, all other behaviours were scored as state events. Behavioural scores were then
192   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 at walls and doors [1]	The number of times the piglet looked at walls and doors
Time watching walls and doors [1]	The amount of times the piglet watched walls and 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

193

Acoustic monitoring and analyses

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

207   A spectro-temporal analysis was performed with custom-written codes using the Seewave R
208   package (Sueur et al., 2008) implemented in R (R Core Team, 2015). We first studied the spectral
209   properties of the remaining background noise of the experimental room (electric noises and
210   remaining low frequency noises from the rest of the building), using 20 examples of 0.5 second
211   fragments. Since the first quartile (Q25) of the normalized spectrum of the background noise was
212   250Hz and the grunts are low frequency vocalisations, we decided to remove all frequencies below
213   200Hz in order to focus on the most relevant frequencies, using a 0.2-8 kHz bandpass filtering ('fir'
214   function). As a consequence, all results presented in this study are on a 0.2-8kHz frequency range

215 and no conclusions on possible frequency components of grunts below this 200Hz threshold can be
216 drawn here. To measure grunt duration, a 5% to maximal amplitude threshold was used ('timer'
217 function). After normalisation, the following spectral parameters were calculated using the
218 'specprop' function (FFT with Hamming window, window length = 512, overlap = 50%): mean
219 (Q50), first (Q25) and third (Q75) quartiles, interquartile range (IQR), centroid and standard
220 deviation (all in Hz). The grunt dominant frequency (in kHz) was also calculated ('dfreq', 50%
221 overlapping FFTs, window length = 512), which is the mean over the grunt duration of the
222 frequencies of highest energy of each window. Frequency peaks were detected and the minimal and
223 maximal peaks were kept as descriptors ('fpeaks' function, window length = 512, peak detection
224 threshold = 10% of the normalized amplitude). Measures of noisiness and entropy of the grunts were
225 assessed using: Shannon entropy (sh), Spectral Flatness (Wiener entropy, sfm) and Entropy (H)
226 [combining both Shannon and Temporal envelop entropy, length = 512, Hilbert envelop). Two vocal
227 scores were used: the logarithm of grunt duration and a built-in spectral vocal score with all spectral
228 parameters (see below). A table describing mean and range of variation of each acoustic parameter in
229 the relevant contexts of the study is available in the supplementary material (table S4).

Statistical analyses

Behavioural and vocal response scores

232 All measures extracted from videos or sound analysis are named parameters throughout the text.
233 The symmetrical distribution of parameters (behavioural on the one hand and acoustic on the other
234 hand) was visually inspected, and linear transformations were computed when necessary to reach
235 symmetrical distribution (see tables 2, 3, 4). When this criteria was reached, Principal Component
236 Analyses (PCA, one for the behavioural analysis and one for the spectral acoustic analysis) were
237 performed using several parameters to build scores ['dudi.pca' function from 'ade4' R package
238 (Dray & Dufour, 2007) and 'inertia.dudi' function to extract the loadings]. These scores were then
239 used as statistical variables. Indeed, PCAs are generally used to reduce the number of variables
240 included in statistical models. It also generates quantifiable global descriptors of behaviours or
241 acoustic structure, since correlated parameters usually load on the same PC (McGregor, 1992). All
242 PCs having an eigenvalue above one were kept and constituted response scores of behavioural
243 ('ReuPCs' and 'CondPCs' in table 2 and 3 respectively) and vocal ('VocPCs', table 4) parameters.
244 Only the duration of grunts was kept separated from the spectral parameters to keep it as a temporal
245 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 (sqrt)	24.177	**-55.843**	-0.435
Nb approaches H (ln)	**47.748**	-30.163	0.578
Time watching H (ln)	**-52.914**	-7.422	25.585
Latency to contact H (ln)	**-64.232**	-0.464	1.688
Nb looks toward H (sqrt)	-7.787	**-43.721**	31.633
Time watching room	-32.048	-13.581	-6.238
Nb looks at walls and doors (sqrt)	3.524	**-72.408**	-2.027
Time in proximal area (sqrt)	**69.96**	-0.156	9.584
Time in distal area (sqrt)	**-46.416**	-12.437	-1.215
Time in contact H (sqrt)	**61.041**	3.586	24.183
Time spent investigating floor	11.868	-7.503	**-42.265**

246

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 (ln)	**80.23**	2.542	-0.112
Time in distal area (ln)	-33.826	8.547	30.789
Number of contacts H (ln)	**78.55**	6.476	2.288
Time in contact H (ln)	**86.625**	0.715	-0.369
Nb looks toward H (ln)	-2	**79.898**	-0.745
Time watching H (ln)	-6.757	**65.67**	-10.325
Nb of zones crossed (sqrt)	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

247

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] (ln)	-0.349	**58.758**	24.236
Max frequency peak[1]	**-43.023**	8.760	-9.537
Mode[2] (ln)	-0.522	**66.248**	19.268
Mean[2] (ln)	**-95.092**	-2.295	2.028
Q50[2] (ln)	**-85.278**	0.280	-0.093
Q25[2] (ln)	**-52.360**	19.327	0.985
Q75[2] (sqrt)	**-88.925**	-4.645	2.309
Centroid[2] (ln)	**-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

249   All statistics were carried out on R (R Core Team, 2015). Linear mixed effect models ['lmer'
250   function, 'lme4' R package (Bates et al., 2014)] were built when tested variables were linear
251   (behavioural and vocal scores, grunt duration) and one binomial generalized mixed effect model was
252   built for binary parameters (occurrence of missed contacts initiated by human during the
253   conditioning). The following subsections describe how models were built for each type of tests. In all

254   models described below, the identity of the replicate ('1' or '2') was used as an interacting fixed
255   factor, since the experiment was run in two identical replicates on two independent groups. The
256   identity of the human ('AH' or 'AV') was used as interacting fixed factor in all models described
257   below, since two experimenters were involved in the positive handling at weaning and in the session
258   of additional positive contacts of the conditioning (but always the same human was attributed to a
259   given piglet). The piglet was used as random factor to take into account the within-subject design.

Isolation/Reunion tests

261   The aim of this part was to test the effect of the positive handling at weaning treatment (H vs. H+
262   piglets) and additional human contacts during sessions of the conditioning on the piglet's reaction to
263   human presence. Since the same Isolation/Reunion test was repeated before and after the
264   conditioning, we used the variable 'Conditioning time' as a two level interacting factor ('before' or
265   'after' conditioning, referred as "Time" in the models) to test the effect of the conditioning. Piglets
266   spacial behaviour and proximity to the human was studied only during the reunion phase with the
267   human that followed the isolation phase. Model_1 was computed:

```
268    Model_1 <- lmer (ReuPCs ~ Treatment*Time + Treatment*Replicate +
269    Treatment*HumanID + Time*Replicate + Time*HumanID + (1 | pigletID), data=
270    data_Behaviour_Reunion).
```

271   Concerning the analysis of vocal behaviour, the isolation phase represents a negative social context
272   for the piglets and may be used as a negative control when monitoring the effect of human presence
273   on vocal expression of emotional states (Villain, Lanthony, et al., 2020). So, the two phases of the
274   test were used to study the three way interaction between treatment (H vs.. H+), phase of the test
275   (isolation vs.. reunion) and time of the conditioning (before vs. after). The following model_2 was
276   computed:

```
277    Model_2 <- lmer (VocPCs ~ Treatment*Phase*Time + Treatment*HumanID + Time*HumanID
278    + Treatment*Replicate + Time*Replicate  + (1 | pigletID/Time/Phase) , data=
279    data_Vocal_Isolation + data_Vocal_Reunion).
```

280   To go further, only the reunion phase was kept and a proximity variable was added. Indeed, the
281   piglet could vocalise either when close to human or away from them and this spatial proximity was
282   demonstrated as an important factor of changes of vocal features (Villain et al. 2020b). Thus, a two
283   level proximity factor was built: either '1' when the piglet was in the proximal area (figure 1) or '0'
284   when it was elsewhere in the room. The following model_3 was computed:

```
285    Model_3 <- lmer (Vocal response score ~ Treatment*Time*InProxArea +
286    Treatment*HumanID + InProxArea*HumanID + Treatment*Replicate +
```

287   InProxArea*Replicate + Time*Replicate + Time*HumanID + (1 | pigletID/Time), data
288   = data_Vocal_Reunion).

Conditioning trials

290   The aim was to study the evolution of human-piglet relationship over the conditioning [the variable
291   'Trial number', used as a continuous variable, referred as "Trial" in the models]. The effect of
292   treatment (positively handled at weaning H+ piglets or non handled H piglets) was tested as an
293   interacting factor with Trial. Trial was also used as a random slope to take into account individual
294   trajectories (Schielzeth and Forstmeier 2009). The following model_4 was built to test the
295   behavioural response scores CondPCs (lmer) and the occurrence of missed contact initiated by the
296   human during a session (presence/absence, binomial model, glmer):

297   Model_4 <- (g)lmer (CondPCs / Missed contact ~ Trial*Treatment + Trial*HumanID +
298   Trial*Replicate + Treatment*Replicate + Treatment*humanID + (1+ Trial |
299   pigletID), (family=Binomial), data= data_Behaviour_Conditioning).

300   For the analysis of vocal response scores, similarly to the Isolation/Reunion test, the piglet could
301   vocalise either when close to the human or away from them. We thus added the proximity factor in
302   the analysis of vocal response variables. The following model_5 was built :

303   Model_5 <- lmer (VocPCs ~ Trial*Treatment*InProxArea+ Trial*HumanID +
304   Trial*Replicate + Treatment*Replicate + Treatment*HumanID + HumanID*InProxArea +
305   Replicate*InProxArea + (1+ Trial | pigletID), data= data_Vocal_Conditioning).

Model validation and statistical tests

307   All linear models were validated by visual inspection of the symmetrical and normal distribution of
308   the residuals. Anovas ('car' R package (Fox & Weisberg, 2011)) were computed on models to test
309   for significant effects of explanatory variables. Following the Anova, when interactions were found
310   significant, post hoc test were run on model interactions, correcting for multiple testing with Tukey
311   contrasts ('emmeans' or 'lstrends' functions from 'emmeans' R package (Lenth, 2016), for
312   categorical or continuous variables respectively). Considering the conditioning time (before or after
313   conditioning), when involved in a significant three-way interaction, this factor was fixed to allow
314   pairwise comparison within each time period as it was not considered relevant to assess the effect of
315   time only. Results of the Anova, model estimates and pairwise post hoc comparisons are reported in
316   the supplementary material (tables S1 and S2 for tests, table S3 for model estimates).

317

Results

Effect of positive handling at weaning and conditioning on piglets' reaction to human presence (Isolation/Reunion tests)

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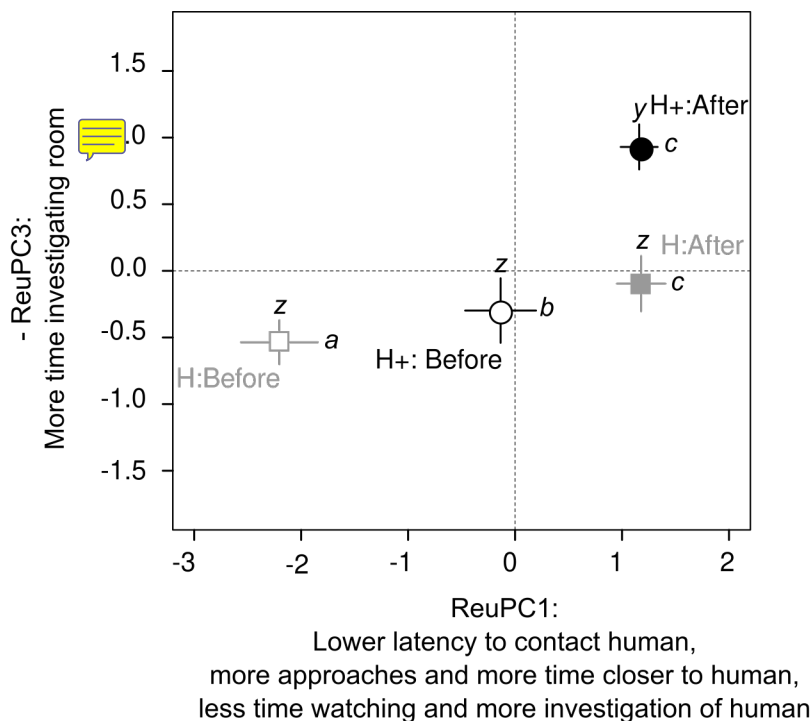

Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean ± SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey squares and H+ : black circles) and time (Before the conditioning: empty elements and After the conditioning: filled elements) on behavioural ReuPC1 (letters a to c) and ReuPC3 (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 at weaning (H+) or not (H) and the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3 (χ^2_1 = 28.0, p < 0.001, and χ^2_1 = 3.7, p = 0.05 respectively, figure 2) but not for ReuPC2 (χ^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

332   that piglets that were positively handled at weaning had a lower latency to contact the human,

333   approached them more often and spent more time close to ~~and~~ investigating the human (ReuPC1)

334   than non handled piglets, before the conditioning. This score increased after the conditioning and no

335   evidence of a difference between treatments after the conditioning was found (figure 2). Post hoc

336   tests on ReuPC3 showed a significant effect of the conditioning time only in piglets that were

337   positively handled at weaning (H+: after – before, t.ratio = 5.2, p < 0.001, H: after – before, t.ratio =

338   2.6, p = 0.06). No difference in ReuPC3 was found between treatments before the conditioning

339   (Before: H – H+, t.ratio = -0.75, p = 0.87), whereas positively handled piglets had a higher -ReuPC3

340   after the conditioning than before (After : H – H+, t.ratio = -3.2, p = 0.009). According to the

341   loadings, this means that after the conditioning, piglets that were positively handled at weaning

342   expressed more investigation of the room after the conditioning than non handled piglets. No

343   evidence of any effect on ReuPC2 was found (table S2).

Piglets positively handled at weaning produce shorter grunts even when no human is present.

346   Using the isolation phase as a negative control we could compare the effect of the phase of the test

347   (Isolation vs. Reunion with the human), taking into account the conditioning time (before or after the

348   conditioning) and the treatment. No evidence of any effect of neither the three way interaction ($\chi^2_1 <$

349   0.62, p > 0.43) nor two way interactions of interest was found (treatment: phase, conditioning

350   time:phase, conditioning time: treatment interactions : χ^2_1 <3.5, p > 0.06, table S2) in any of the

351   scores.

352   Regardless of the treatment, single effects of the phase of the test were significant for grunt

353   duration and all AcPCs (χ^2_1 > 6.6, p < 0.01, table S1). During the reunion phase with the human,

354   grunts were shorter (estimates of log(duration)[95% CI] : -1.32[-1.37;-1.26] vs. -1.06[-1.12;-1.00]),

355   had a higher frequency range, higher bandwidth and a higher noise component (-VocPC1:

356   0.78[0.48;1.08] vs. 0.34[0.03;0.66]), were higher pitched (VocPC2: -0.18[-0.36;0.01] vs. -0.46[-

357   0.65;-0.28]) and their spectrum had a higher skewness and kurtosis (VocPC3: -0.25[-0.37;-0.14] vs. -

358   0.11[-0.23;0.01]), compared to the isolation phase.

359   Regardless of the phase of the test, single effects of treatment were found for grunt duration and -

360   VocPC3 (χ^2_1 = 5.5, p = 0.02 and χ^2_1 = 4.9, p = 0.03 respectively, table S2). Grunts produced by

361   positively handled at weaning piglets were shorter (estimates of log(duration)[95% CI]: -1.25[-1.32;-

362   1.19] vs. -1.12[-1.2;-1.1], table S3), and differed in -VocPC3 scores, describing the shape of the

363   frequency spectrum (estimates of -VocPC3[95% CI]: -0.29[-0.43;-0.14] vs. -0.07[-0.22;0.08], table
364   S3), than grunts produced by non handled piglets.

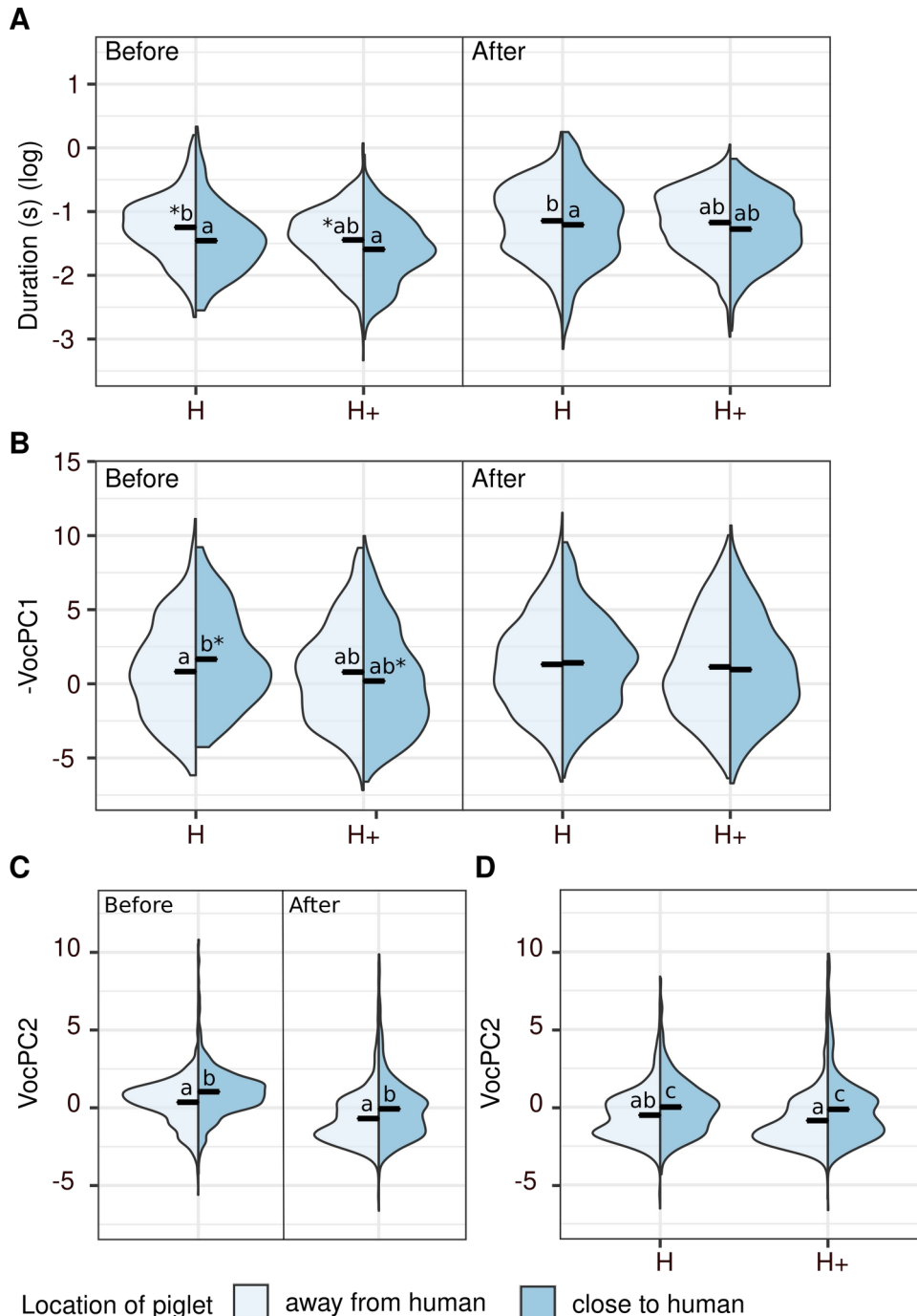365   *Positive handling and conditioning affect vocal reactivity to human proximity.*

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 significant three way interaction between treatment, conditioning time and location on grunt duration (A) and on the first vocal score -VocPC1 (B). (C,D) Results of post hoc tests following significant two way interactions between conditioning time and location (C) and between treatment and location (D) on the second vocal score VocPC2. Values with no common letters differ significantly. 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).

366   During the five-minute reunion, the piglet was scored either as close to the human or away from
367   them. The three way interaction of the conditioning time, the treatment and the location was
368   significant for grunt duration, -VocPC1 and VocPC3 ($\chi^2_1 > 4.9$, $p < 0.03$). Post hoc tests revealed
369   that grunts produced closer to the human were shorter than the ones produced further away, but only
370   in piglets that were not positively handled at weaning, effect being stronger before the conditioning
371   than after it (H piglets: away – close, z.ratio = 6.3, $p < 0.001$ before and z.ratio = 4.1 $p < 0.001$ after
372   the conditioning; H+ piglets: away – close z.ratio < 1.98 p > 0.19, figure 3A). -VocPC1 was higher,
373   i.e. grunts had a higher frequency range, bandwidth and were noisier when produced closer to the
374   human than further away, but only in non handled piglets and before the conditioning (H piglets:
375   away – close, z.ratio = -3.34, p = 0.005 before and z.ratio = -1.23 p = 0.61 after the conditioning; H+
376   piglets: away – close, z.ratio < 0.36 p > 0.21, figure 3B). For VocPC2, the three way interaction did
377   not reach significance ($\chi^2_1 = 3.3$, p = 0.07), so only subsequent two way interactions were
378   considered (post hoc tests on the three way interaction can be found in supplementary, tables S1 to
379   S3). For VocPC2, significant two way interactions were found between the conditioning time and the
380   location ($\chi^2_1 = 10.3$, p = 0.001) on the one hand, and between the location and the treatment ($\chi^2_1 =$
381   4.2, p = 0.04) on the other hand. Post hoc tests revealed that grunts produced closer to the human had
382   a higher VocPC2, meaning they had a higher pitch, effect being stronger before the conditioning than
383   after (before: away – close, z.ratio = -6.12, p < 0.001; after: away – close, z.ratio = -2.88, p = 0.004,
384   figure 3C). The increase in VocPC2 with the location was greater for non handled piglets than
385   positively handled piglets (H piglets: away – close, z.ratio = -5.54, p < 0.001; H+ piglets: away –
386   close, z.ratio = -3.82, p = 0.001, figure 3D). The last two-way interaction of interest between the
387   conditioning time and the treatment did not reach significant level ($\chi^2_1 = 0.80$, p = 0.37). For
388   VocPC3, post hoc tests did not reach significant levels (|z.ratio| < 2.3 p > 0.09 for any comparison) .

389

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

The conditioning increases behavioural proximity to the human in all piglets.
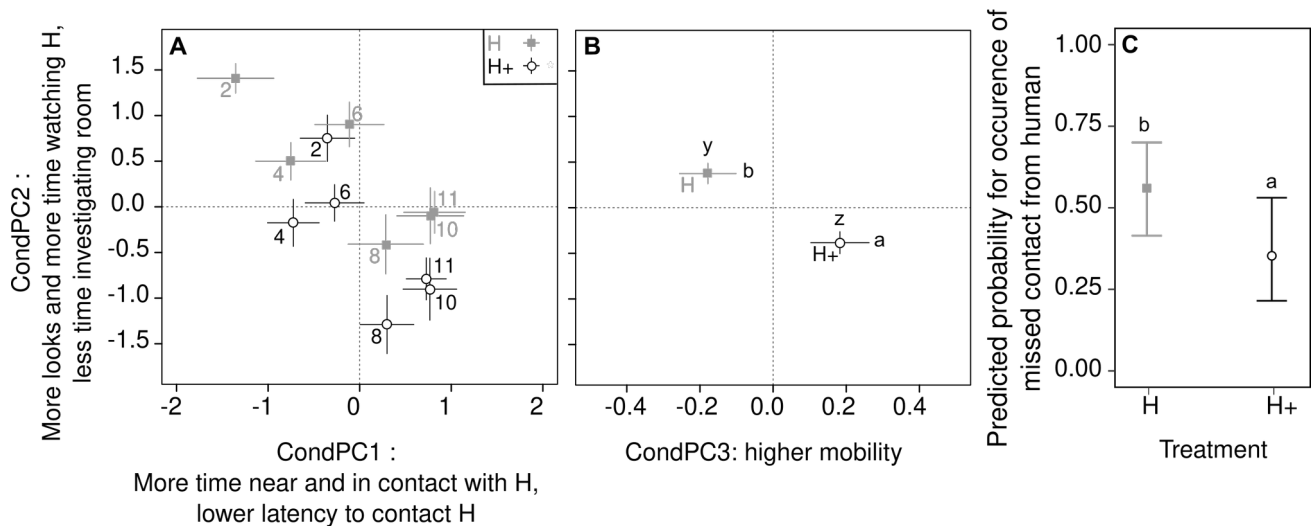
Figure 4: Behavioural variation of responses of piglets according to the sessions of additional positive contacts of the conditioning (A), and to the treatments (B, C). (A, B) Mean ± SE per group, numbers in (A) refer to the trial number of the conditioning. Higher CondPC1 and lower CondPC2 over time (single effect of trial number, A) and well as higher CondPC2 scores in H piglets than H+ piglets regardless of time (single effect of treatment, B). Higher CondPC3 and lower CondPC2 scores of H+ piglets compared to H piglets (single effect of treatment, B). (C) Mean estimates ± 95% confidence interval from the generalized mixed effect model. Lower probability of occurrence of missed contact by the human in H+ piglets (significant single effect of treatment following non significant interaction with trial number). 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

407   lower for positively handled piglets than non handled ones (χ^2_1 = 9.57, p = 0.002, figure 4C), with no

408   interaction with the trial number (χ^2_1 = 0.22, p = 0.064).

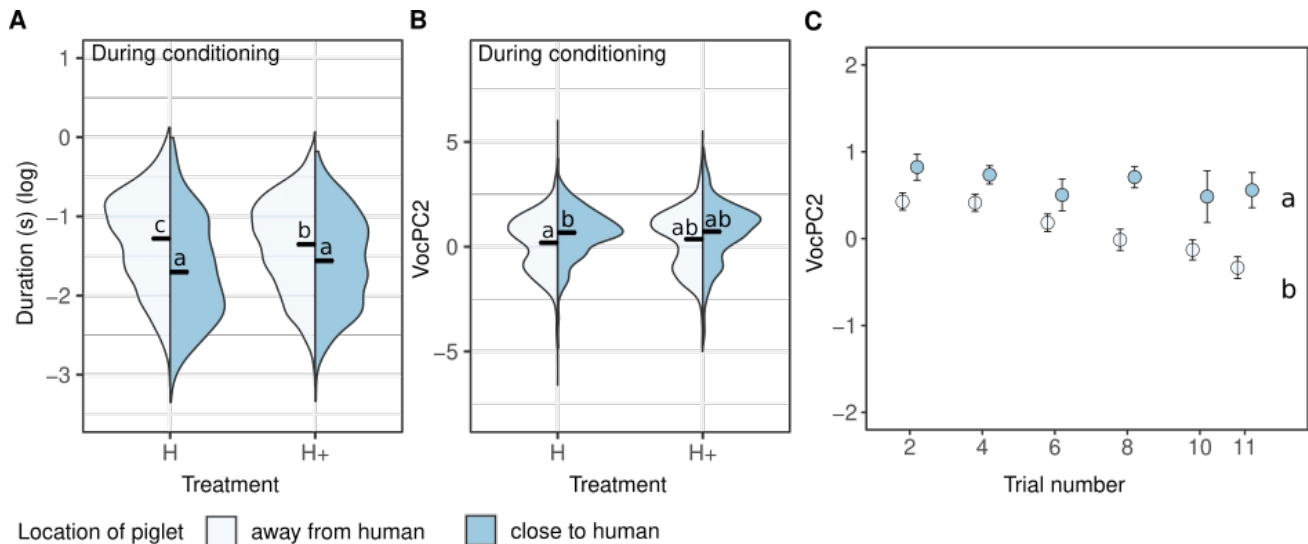### *Additional positive contacts trigger shorter and higher pitch grunts in all piglets.*

Figure 5: 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. Values with no common letters differ significantly (difference between groups: A, B or slopes: C). Full statistical report is available as supplementary material (tables S1-S3).

410

411   During the sessions of additional positive contacts of the conditioning, the three-way interaction

412   between the trial number, the treatment and the location was not significant for any of the vocal

413   scores (χ^2_1 < 0.18, p > 0.67), allowing the analysis of the two way interactions of interest. The

414   interaction between treatment and the trial number was not significant for all vocal scores (χ^2_1 < 2.5

415   p > 0.11). Grunt duration decreased over time and independently from the treatment (trial

416   number:replicate interaction, χ^2_1 <5.3 p = 0.02, slope estimate -0.03[-0.04;-0.01] for the lower slope,

417   table S1 and S3). However, independently from the trial number, grunt duration was lower when

418   piglets were located close to the human and this effect was stronger in non handled piglets than

419   positively handled piglets (treatment:location interaction: χ^2_1 = 15.8 p < 0.001, away vs.. close, H

420   piglets: z.ratio = 10.2 p < 0.001, H+ piglets: z.ratio = 6.86 p < 0.001, figure 5A). -VocPC1 and

421   VocPC2 decreased over time but remained higher when piglets were located close to the human (trial

422   number: location interaction, χ^2_1 = 3.97 p = 0.05 and χ^2_1 = 6.1 p = 0.01 respectively for -VocPC1 and

423   VocPC2). According to the loadings, this means that the frequency range, bandwidth and noisiness

424   of grunts (-VocPC1) as well as the pitch (VocPC2) decreased over the conditioning when piglets

425   were located away from the human but remained high when piglets were close (slope comparison

426   away – close, -VocPC1 : z.ratio = -1.80 p = 0.07, VocPC2 : z.ratio = -2.34 p = 0.02, figure 5C).

427   Additionally, VocPC2 was higher when piglets were close to the human in non handled piglets

428   (treatment:location interaction, χ^2_1 = 7.6 p = 0.005, pairwise comparisons away vs. close, in H:

429   z.ratio = -4.9 p z 0.001 and in H+: z.ratio = -2.0 p = 0.21), meaning that non handled piglets

430   produced higher pitched grunts when closer to the human (figure 5B).

431   ## Impact of human identity on piglets behaviour and grunt structure

432
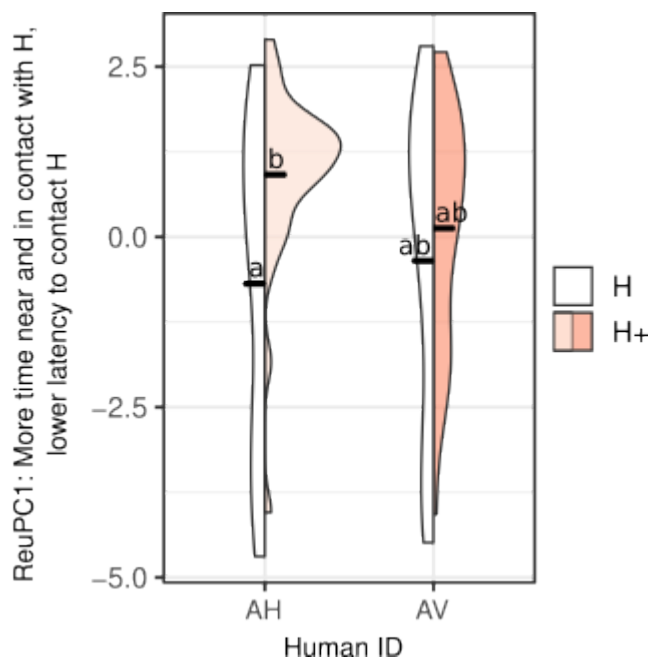
Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Values with no common letters differ significantly. Full statistical report is available as supplementary material (tables S1 and S2 for statistical tests, table S3 for model estimates).

433   Since half of the piglets had been assigned to one human experimenter and the other half to another

434   one, the identity of the human was included in the model. This allowed to test interactions between

435   the identity of the human and the treatment of positive handling at weaning on the one hand and the

436   conditioning time on the other hand.

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

438   identity was significant for the first behavioural proximity score (ReuPC1, χ^2_1 = 6.01, p = 0.01) but

439   not the others (ReuPC2 and ReuPC3 (χ^2_1 < 1.98, p > 0.16, table S1). The effect of treatment on

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

441   < 0.001, figure 6). When the human 'AV' handled the piglets, for which ReuPC1 scores exhibited

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

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

444   considering the reunions of the conditioning ($\chi^2_1 < 1.32$, p > 0.25 for all CondPCs, table S1).

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

446   considering the reunions of the Isolation/Reunion test (ReuPCs, $\chi^2_1 < 0.642$, p > 0.42, tables S1),

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

448   (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

449

450   Considering the vocal scores, no effect of human identity was found on VocPC1 during the

451   Isolation/Reunion tests but -VocPC1 was higher when the human 'AH' was in the room during the

452   reunion periods of the conditioning (table 5), meaning the frequency range and the bandwidth of the

453   grunt were higher when the human 'AH' interacted with the piglet compared to the human 'AV'.

454   VocPC2 was higher when the human 'AH' was in the room during the Isolation/Reunion tests (table

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

456   additional positive contacts of the conditioning in interaction with the location of the piglet (χ^2_1 =
457   11.9, p = 0.001): VocPC2 increased when piglets were located close to the human but this increase
458   was significant only for the human 'AV' and not for 'AH' (table 5). VocPC3 was not different
459   between humans during the reunions of the Isolation/Reunion tests but, over the conditioning,
460   VocPC3 changed differently when piglets were handled by the human 'AH' or 'AV', as showed by
461   the significant interaction between trial number and human identity (χ^2_1 = 8.0, p = 0.005): the
462   skewness and kurtosis of grunts decreased over the conditioning when 'AH' was interacting with the
463   piglets, but not 'AV' (see slope estimates, table 5). No evidence of any effect of human identity was
464   found on grunt duration neither during the Isolation/Reunion tests nor during the sessions of
465   additional positive contacts of the conditioning (table S1).

Discussion

466

467   In this study, familiarity to a human and human-animal interactions were experimentally modified
468   in weaned piglets to study the establishment of a positive HAR and test whether grunt structure could
469   reflect a positive HAR. A positive conditioning paradigm, using additional positive contacts from a
470   human as a reward, allowed to compare the behavioural changes over time in piglets previously
471   positively handled at weaning or not. Two types of sessions were studied: a standard
472   isolation/reunion tests with the human, carried out before and after conditioning, during which the
473   human remained silent and did not interact with the piglet, and sessions of the conditioning, during
474   which the human interacted with the piglets, providing additional positive contacts, as long as the
475   piglets stayed close to the seated human. Behavioural data were collected to describe the positive
476   HAR. Grunts produced during the tests and sessions were collected and their spectro-temporal
477   structure confronted to the behavioural data, with the hypothesis that vocalisation structure may
478   reflect the quality of HAR, though vocal markers of positive emotions. Firstly, the discussion will
479   focus on the behavioural validation of the establishment of a positive HAR. Secondly, behavioural
480   and vocal expression will be confronted to discuss grunt spectro-temporal structure as indicator of
481   the quality of HAR. Last, we will discuss perspectives regarding the effect of human identity on the
482   establishment of a positive HAR.

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

483

484

485   The standard reunion test with the human before the conditioning showed first that the treatment of
486   positive handling at weaning succeeded in creating two different levels of human-piglet relationship

487   (H and H+), as positively handled piglets expressed a higher attraction toward the human than non

488   handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al.,

489   2020). Second, this test showed that the conditioning increased the behavioural proximity toward the

490   human of both positively handled and non handled piglets so that non handled piglets expressed a

491   similar attraction toward the human as positively handled piglets. These results are in line with the

492   behavioural results of the sessions of additional positive contacts. The analysis of piglets' behaviour

493   every second sessions of the conditioning showed that, although positively handled and non handled

494   piglets started with different degree of proximity toward the human (trials 2 and 4, CondPC1), then,

495   over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human

496   (CondPC1) and avoided less the human when the latter attempted to interact with them. So it seems

497   that the conditioning process allowed non handled piglets to compensate the lack of positive

498   handling before the conditioning and develop a similar proximity toward the human. Two minute

499   daily sessions of additional positive contacts changed positively the perception of the human for the

500   piglets, and thus their willingness to interact with them. Since no evidence of any interaction

501   between time and treatment was found, no conclusion on differential developmental trajectories

502   between treatments can be drawn, but a parallel development of the human-piglet relationship in

503   both groups, when considering the proximity.

504   Beside behavioural proximity, piglets that were positively handled at weaning expressed more

505   exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also

506   observed during the sessions of additional positive contacts of the conditioning: positive handled

507   piglets started with a higher score associated with investigation than non handled piglets (CondPC2)

508   and it held over the conditioning. Piglets that were positively handled at weaning also expressed a

509   higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an

510   expression of natural foraging and disinterest from human contact, which may be a sign of positive

511   welfare (Weerd & Day, 2009). In addition, this could also be interpreted in terms of attachment to

512   the human. Indeed, attachment to a human may facilitate exploration of novel environments or

513   objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may

514   provide an environment secure enough for the piglets to explore their environment in the presence of

515   the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al.,

516   2009).

517   Overall, the behavioural monitoring showed that two minute sessions of positive additional

518   contacts per day are sufficient to increase proximity to a human to similar levels as when piglets were

519   previously familiarised for 2 weeks, even when piglets experienced social isolation. But it did not

520   allow the non handled piglets to express natural exploratory behaviours as the positively handled

521   piglets. We hypothesize a sequential establishment of a positive HAR over time: firstly with a

522   decrease of attentive state and an increase in proximity and accepted contacts, and secondly with a

523   disinterest of human contacts and the expression of natural foraging behaviour. The latter may

524   require a higher exposure time.

525   In the next paragraph we discuss to what extent changes in grunt spectro-temporal structure may

526   reflect behavioural changes linked to the positive HAR over time.

Links between vocal expression and positive HAR

A positive HAR is reflected by shorter grunts in presence and absence of a human

529   The social isolation phase of the Isolation /Reunion test, before any human entered the room, was

530   associated with longer, lower pitched grunts with a downshifted frequency spectrum, whereas the

531   reunion with a static human changed grunts structure to shorter, higher pitched with an upshifted

532   frequency spectrum and this was observed in both handled and non handled piglets (H or H+) as well

533   as before and after the conditioning. In terms of emotional indicators, similar changes in acoustic

534   features of grunts were found in studies focusing on vocal markers of valence in pigs (Briefer et al.,

535   2019, 2022; Friel et al., 2019; Villain, Hazard, et al., 2020), meaning that the reunion with a human,

536   after a period of social isolation would be perceived as positive. However, this modulation of grunt

537   structure was observed regardless of piglet experience with the human. It is possible that the reunion

538   with an either neutral or familiar human, releasing piglets from total isolation could be perceived as

539   positive by the piglets, as suggested in previous studies (Villain, Lanthony, et al., 2020).

540   In addition, and surprisingly, positively handled piglets produced shorter grunts than non handled

541   piglets regardless of human presence. This was previously shown in another context (anticipation of

542   (pseudo)social events independently from the type of partner) in the same groups of piglets (Villain,

543   Hazard, et al., 2020). This may show that the period of positive handling at weaning modulated vocal

544   expression in the long term, as this result was found both before and after the conditioning. On the

545   one hand, a positive HAR establishes through successive positive experiences (Rault et al. 2020)

546   and, on the other hand, HAR may have long term effects on behavioural expressions, as suggested by

547   Brajon et al. (2015) using cognitive bias tests. We can thus hypothesize this may also be reflected in

548   the way piglets vocalise, in general. In that case, we may have evidence of expression of another

549   category of affect, moods, and not only emotional expression. Indeed, as suggested by Schnall
550 (2010), although emotions are short-term affects triggered by an external stimulus, moods, on the
551 other hand, may be experienced on a longer term and may not be attributable to a specific stimulus.
552 Although emotions and moods do not rely on the same time scale, they may interact with one
553 another, and more studies are needed to understand their effects on vocal expression.

A positive HAR affects vocal reactivity toward a static human

555   In a previous study, we showed that pigs vocalizing close to a human that previously had provided
556 repetitive additional positive contacts produced shorter and higher pitch grunts, compared to when
557 vocalizing away from the human (Villain, Lanthony, et al., 2020). Using the same type of test with
558 positively handled at weaning and non handled piglets, before or after conditioning sessions with
559 positive interactions, we can test the effect of positive handling on this modulation of grunt structure.
560 Similarly to the previous study, during the standard reunion test (no contact from the human), piglets
561 produced shorter and higher pitched grunts with an upshifted frequency spectrum when close to the
562 human. It has to be noted that this effect was 1) stronger in previously non handled piglets than
563 positively handled at weaning piglets and 2) stronger before the conditioning than after. In other
564 words, the more familiar with the human associated with positive handling, the less reactive to
565 human proximity.

566   These results may be interpreted according to the behavioural results we described earlier (fig. 2).
567 We described that the proximity to the human was first increasing at the beginning of positive
568 handling experiences (see H piglets, before vs. after conditioning) before reaching a maximum (see
569 H vs. H+ piglets after conditioning) and that the most familiar piglets showed more exploratory
570 behaviours (H+ after conditioning). The acoustic results during the standard reunion mirror the
571 behavioural results from the same test. The least familiar piglets would vocally express the
572 exploration of a neutral and static human and, as the familiarity with the human increases, the human
573 may become part of their environment, explaining the lack of vocal reactivity when close to the static
574 human.

575   In addition, we may also be facing ceiling effects in terms of vocal flexibility, which could also
576 partly explain these results. We showed that positively handled piglets generally produce shorter
577 grunts than non handled piglets, and that the shape of the frequency spectrum of these grunts was
578 different. So the structure of their calls, in general is different. According to the source-filter theory
579 of vocal production, vocal flexibility is constrained by the dimensions and functioning of the vocal

580   apparatus (lung capacity, characteristics of the vocal folds, length and shape of the vocal tract, see
581   (Taylor & Reby, 2010) and (Titze & Martin, 1998)). It is possible that the positive HAR developed
582   by the positively handled piglets may have change their grunts structure to an extent that vocal
583   flexibility is no longer quantifiable in the experimental design of this study.

Providing rewarding additional positive contacts triggers short and high pitched grunts

586   Contrary to the standard reunions with a static human, the human actively interacted with the
587   piglets during the sessions of the conditioning, providing contacts and producing speech as long as
588   the piglets remained close to the human. During these sessions and contrary to the standard reunions,
589   grunts produced close to the human were shorter and higher pitched, regardless of the trial number of
590   the conditioning and treatment. Although these effects were stronger in non handled piglets than
591   positively handled piglets, they remained over time. We describe here two types of vocal reaction to
592   human proximity, depending on the human behaviour. On the one hand, time decreased vocal
593   reactivity to human proximity during a standard reunion with a static human. On the other hand, no
594   evidence of a decrease in vocal reactivity to human proximity was found during sessions of
595   additional positive contacts. This would mean that positive interactions with piglets consistently
596   triggers the production of shorter and higher pitch grunts. These changes may be explained by the
597   expression of a higher arousal state experienced by the piglets while being positively handled.
598   Indeed, in the context of these sessions, the piglet could choose to approach and stay close to the
599   human, which will provide positive contacts systematically. So the piglet may anticipate to receive
600   positive contact and systematically being rewarded. When close to the human, observed changes in
601   frequency distribution of grunts (increased pitch and upshifted frequency spectrum) are known to be
602   markers of arousal (in the negative state in multiple mamalian species (Briefer, 2012, 2020) and pigs
603   (Linhart et al., 2015)). In addition, these spectral changes were also associated with shorter grunts.
604   Although the duration of grunts is associated with the valence of a situation, the duration may also be
605   an indicator of positive arousal. This hypothesis has to be taken precociously since no additional
606   control of arousal could be done in the present study.

607   This working hypothesis may explain the decrease in vocal reactivity to human proximity observed
608   during the standard reunion test as the HAR becomes more positive. Indeed, before the sessions of
609   the conditioning, positively handled piglets were habituated to a human interacting positively when
610   present whereas non handled piglets were not, hence, during the first standard reunion test, when the
611   human is present but do not interact with the piglet, positively handled and non handled piglets may

612   have diverging expectations regarding the presence of the static and silent human. As positively

613   handled piglets received positive contacts every time they were in the presence of the human, they

614   may have expected positive contacts when approaching and experienced an absence of reward during

615   the test. This has already been hypothesised in piglets deprived from human voice during interactions

616   after a period of habituation to it (Bensoussan et al. 2020). On the contrary, piglets that were not

617   positively handled at weaning never experienced additional positive contacts and being close to a

618   human, having the possibility to investigate them may be some kind of reward after the period of

619   total isolation. After the conditioning, piglets from both treatments were conditioned to receive

620   additional positive contacts and both groups had experienced a first standard reunion test, so they

621   may both experience an absence of reward during the test, which may explain a lower reaction to

622   human proximity, and thus fewer changes on grunt spectro-temporal features.

623   Last, we can raise the question whether changes in grunt structure in reaction to rewarding positive

624   contacts may also be associated with a specific human-pig communication. In other domestic

625   species, owner directed vocalisations has been shown (in cats, reviewed in (Turner, 2017); in dogs

626   (Gaunet et al., 2022)). In addition, studies have found similar socio-communicative behaviours

627   toward a human in socialized pigs and dogs (Gerencsér et al., 2019). Hence, we may profit from

628   testing the existence of human directed vocalisations in pigs, as consequences of their socio

629   communicative abilities.

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

632   We found that the identity of the human had effects on behavioural and vocal response scores.

633   Piglets that were handled by the human 'AH' had higher values of behavioural proximity (ReuPC1)

634   than piglets handled by the human 'AV' during reunion test after a period of isolation. This effect

635   was not found during conditioning sessions. The effect of the human did not interact with the

636   conditioning time, leading to the conclusion that the difference between the two experimenters may

637   have established during the period of positive handling at weaning, prior to the conditioning.

638   Additionally, when the human 'AH' was in the room, piglets produced grunts with a more upshifted

639   frequency spectrum and a higher pitch than when the human 'AV' was in the room. If upshifted

640   grunts may be a indicator of positive higher arousal, then we may conclude that 'AH' was more

641   likely to trigger higher positive states than 'AV'. Interestingly, the human identity and the spatial

642   proximity had different effects on piglets grunts during sessions of additional positive contacts but

643   not when the human was static during the standard reunion test. Hence, it is possible that the way one

644   human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering

645   positive emotions and thus modifications of grunt structure. Several evidence exists in the literature

646   that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al.,

647   2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We

648   may question the efficiency of different human features to generate a positive HAR. In our study,

649   both humans that interacted with the piglets wear exactly the same clothes and standardized their

650   tactile interactions toward the piglets before starting the study, and agreed on the rhythm and types of

651   sounds (words, intonation) to use, to minimise generating variability although no systematic controls

652   of the human behaviour or spectral feature of voices were performed here. It thus remains unclear

653   whether experimenters interacted differently or if they were initially perceived differently by piglets.

654   Our results show that the identity of the human may modulate piglet proximity and vocal behaviour

655   but the design of this experiment does not allow to find the causes of these observations (behaviour,

656   voice characteristics, or even odour profile). Thus, more studies of human features that are most

657   likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In

658   addition, studying human-piglet relationship in a more systematic way, as in other domestic species,

659   for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech

660   (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies

661   of interspecific relationships. However, the influence of human identity did not modify the general

662   outcomes of our study, but only decreased some effects, suggesting that this variability does not

663   modify the main results, but should be considered in future studies.

664

665   To conclude, we showed that degrees of familiarity toward a human could be reflected in the way

666   piglets vocalise in their presence, and out of it. We also showed that the spatial proximity toward a

667   human providing additional care could change the acoustic structure of piglet grunts. These changes

668   are likely to be linked to positive and more intense emotional states than when piglets are further

669   away from the human. However, it is still unclear whether the changes in grunt structure could also

670   be linked to human-animal communication and more studies are needed to determine it. We did also

671   show that the identity of the human may be of importance, and may generate vocal changes during

672   additional positive contacts that were not associated with changes in behaviour of the human. More

673   systematic studies of human behaviour along with pig behaviour during the human-animal

674   interactions would be needed to have a better understanding of the evolution of HAR, especially

675   interactive interspecific communication as well as providing new procedures to promote positive

676   welfare. We suggest that analysing vocalisations structure may be a good tool to assess the quality of

677   human-pig relationship and help monitor the establishment of a positive HAR.

Authors contributions

679   Conceived and designed the experiment (A.V., C.T., C.N.). Performed the experiment (A.V., C.G.).

680   Collection and processing of the acoustic and behavioural data (A.V., C.G.). Statistical analyses

681   (A.V.). Contributed to the writing of the manuscript (A.V., C.T., C.N.).

Acknowledgments

Data availability

690   The datasets used for the study are available at (Villain et al., 2022). The folder contains all datasets

691   and a readme to match the type of analysis to the proper dataset. We have made sure to report in the

692   main text of the article which R libraries and which functions in these libraries we used. All formulas

693   of the statistical models are explicit in the text to facilitate transfer of information and replicate the

694   analysis. All libraries are open source as well.

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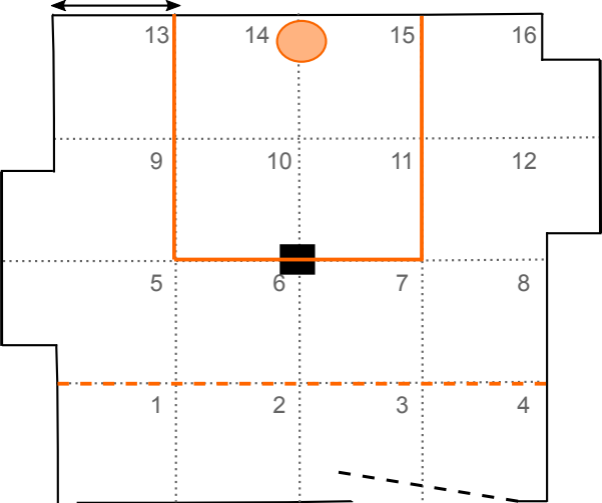
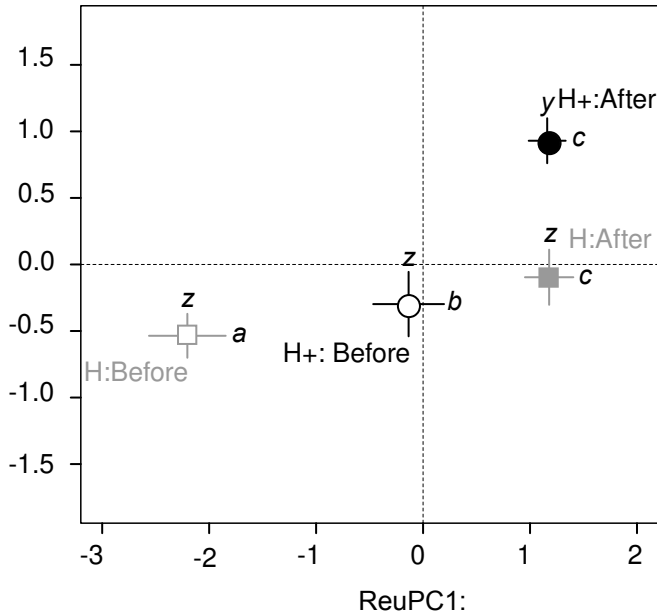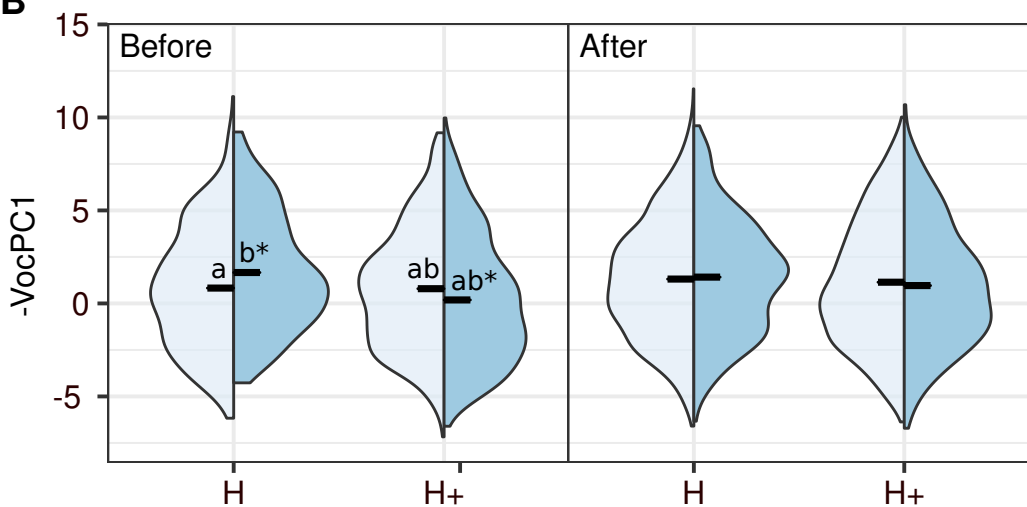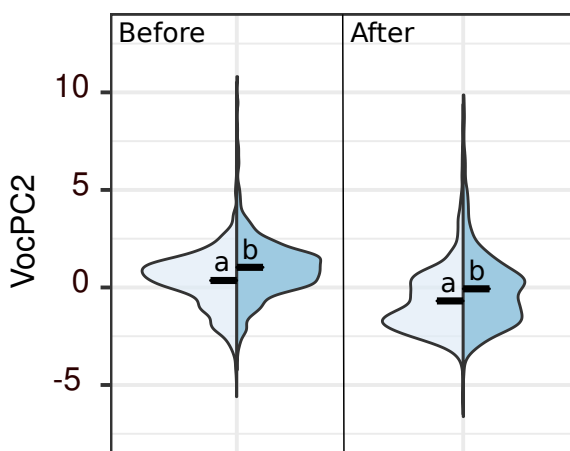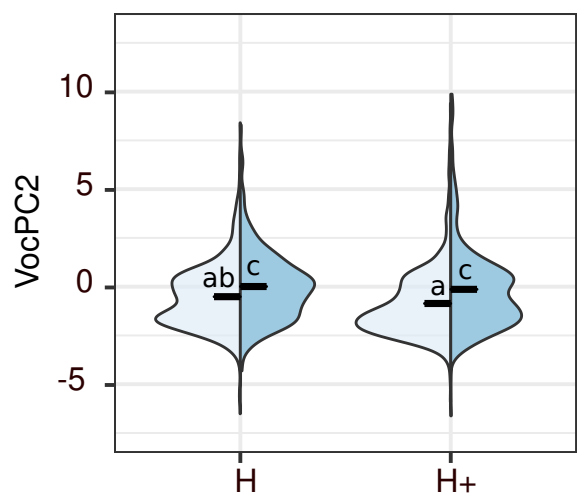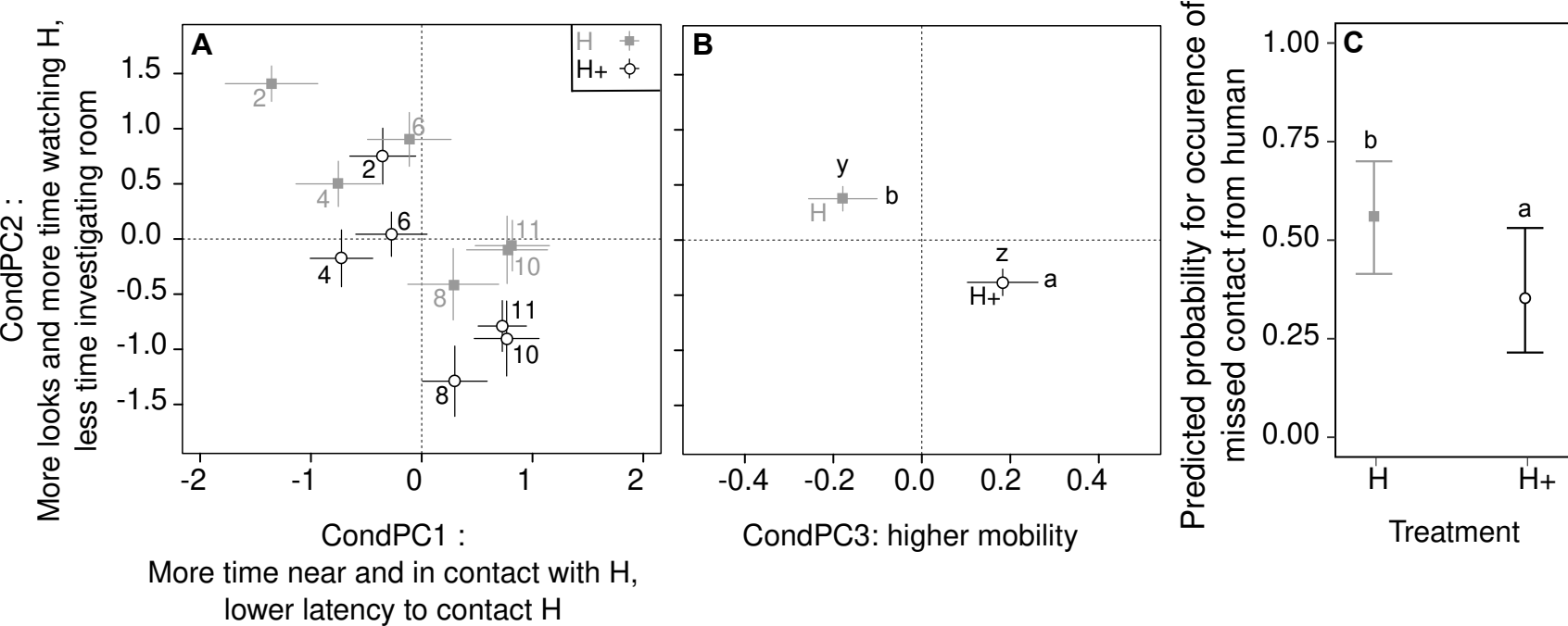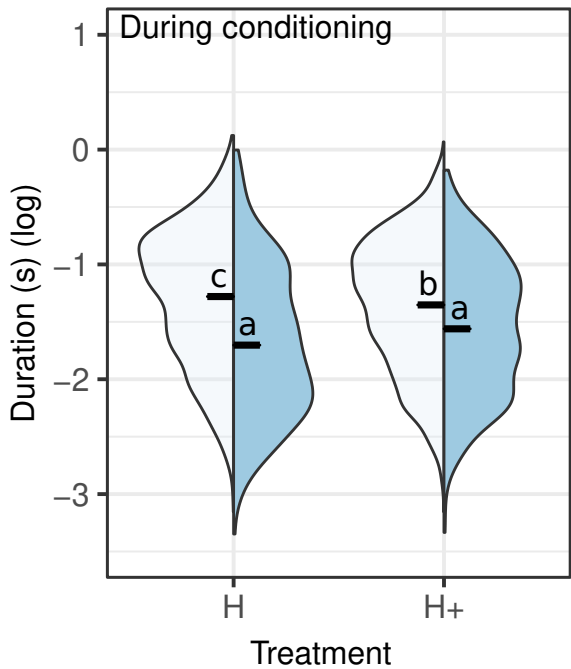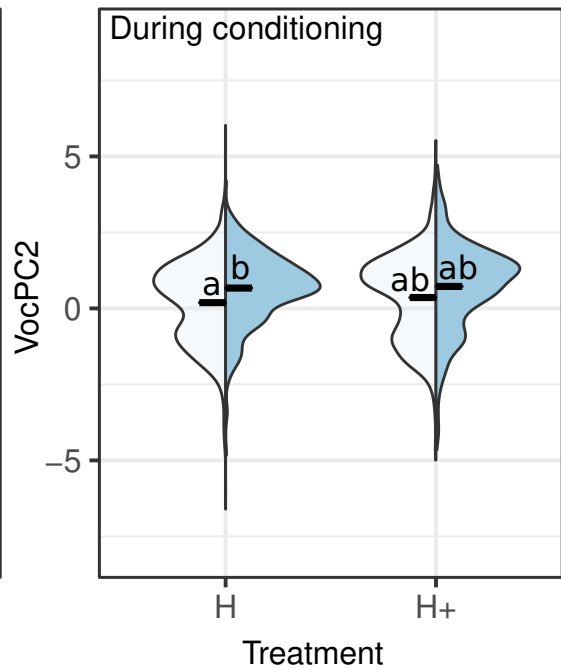| ↔	65.5cm
■	Location of the microphone
⬚	Virtual zones for behavioural scoring
🔴	Location of the human
▭	Proximal area
▭	Distal area

- ReuPC3:
More time investigating room

ReuPC1:
Lower latency to contact human,
more approaches and more time closer to human,
less time watching and more investigation of human

Location of piglet: away from human, close to human

A During conditioning

Duration (s) (log) vs Treatment (H, H+)
- H: c, a
- H+: b, a

B During conditioning

VocPC2 vs Treatment (H, H+)
- H: a, b
- H+: ab, ab

C

VocPC2 vs Trial number (2, 4, 6, 8, 10, 11)
- a
- b

Location of piglet: □ away from human ■ close to human