

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

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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

20 animal biology and emotional states (social, spatial and postural behaviours, physiological and
21 cognitive states). Growing evidence shows that acoustic features of animal vocalisations may be
22 indicators of emotional states. Here, we tested the hypothesis that vocal structure may indicate the
23 quality of HAR. At weaning, 30 piglets were positively handled by an experimenter who talked to
24 and physically interacted with them three times a day, while 30 other piglets only received the
25 contact necessary for proper husbandry. After two weeks, we recorded the behaviours and
26 vocalisations produced in the presence of the static experimenter for five minutes. We repeated this
27 test two weeks later, after a conditioning period during which human presence with additional
28 positive contacts was used as a reward for all piglets. We hypothesized this conditioning period
29 would lead to a positive human-piglet relationship for all piglets. As expected, piglets that were
30 positively handled at weaning expressed a higher attraction toward the experimenter, and, after the
31 conditioning, piglets that were not positively handled at weaning expressed a similar level of
32 attraction than the positively handled ones. Piglets positively handled at weaning produced shorter
33 grunts than the other ones, [regardless of the context of recording, which may indicate a more positive](#)
34 [affect. During reunions with the static experimenter, a more positive HAR was associated with a](#)
35 [decrease in vocal reactivity to human proximity. However, during reunions with the experimenter](#)
36 [providing additional positive contacts and over the conditioning, proximity to the human](#)
37 [systematically triggered shorter and higher pitched grunts, indicator of positive a emotional state.](#)
38 Results first show that changes in vocal structure are consistent with indicators of positive states in
39 the presence of a human. [Second, these changes are stronger when the human positively interact with](#)
40 [the piglets, supposedly emphasizing a higher positive arousal state during these interactions. We](#)
41 [show that vocalisation structure may be a promising indicator of the quality of human-pig](#)
42 [relationship.](#)

43 **Introduction**

44 The process of domestication was conducted to shape physiology and morphology of domestic
45 animal species, but also their behaviour. It notably has shaped interspecific interactions between
46 human and non-human animals, by improving animals' capacity to use human signals to adapt their
47 behaviour both decreasing fearfulness toward humans and increasing attention toward humans
48 (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is
49 important for animal welfare. Therefore, studying human-animal interactions and their consequences
50 to understand the mechanisms of emergence and maintenance of a positive human-animal
51 relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three
52 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, Lanthony, et al., 2020) 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, some vocalisations type 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. Second, vocalisation structure is known to carry markers of the emotional states in several bird and mammal species (Briefer, 2012, 2020) and markers of emotional valence (positive versus negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Since positive or negative HAR is likely to affect the emotional state of animals, it is likely that it may be reflected in the structure of the produced vocalisations.

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;

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).

109 **Methods**

110 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.

117 Subjects and housing conditions

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

122 One piglet had to be excluded from our sample size to receive care/medication due to health issues
123 independent from the experiment. From weaning at 28 days of age, piglets from the same litter and
124 having similar weight (<1 kg difference) were housed by three in a 1.2 x 1.3m pen on plastic
125 duckboard. Wooden panels were used to visually isolate pens. One metal chain per pen was used for
126 enrichment. Food and water were available *ad libitum*. Artificial lights were turned on from 8:00 to
127 17:00 and temperature was maintained between 26 and 27 °C. The experiment was carried out in two
128 replicates and two identical rearing rooms were used (5 pens per room per replicate).

129 Treatment: positive handling at weaning

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

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

136 - **Positively handled piglets piglets (H+ piglets):** Experimental piglets from the 10 other rearing
137 pens, housed in another room, received the same daily care given by the same stockperson as for H
138 piglets. They additionally received repeated sessions of additional human contacts. Each pen of three
139 piglets received 29 sessions of 10 minutes, from day 28 (weaning) until day 39, occurring five days a
140 week. Three sessions per day were performed (except on the day of weaning during which only two
141 were done with a two-hour break in between). Each session took place in the rearing pen and the
142 order of the interventions in the pens was balanced across days. The handling procedure, using gentle
143 tactile contacts is described in supplementary material of Villain et al. (2020) and was similar to
144 Tallet et al. (2014). Two experimenters performed these sessions (both women, both between 1.70-
145 1.73 m tall, with a balanced number of pens attributed to each of them). The experimenters wore the
146 same blue overalls and green boots each time they interacted with the piglets. The experimenters
147 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.

152 Conditioning: sessions of additional positive contacts with 153 (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 expected 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).

176 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).

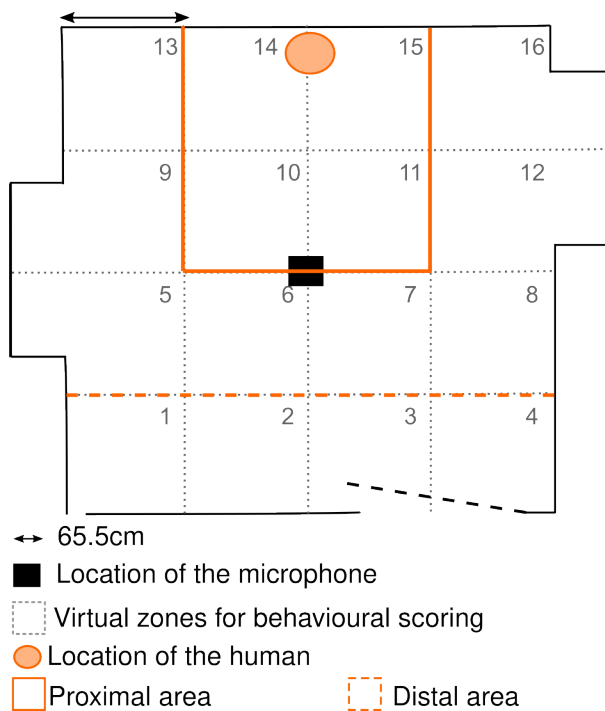


Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1, 2, 3, 4) were defined, using the location of the human as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

194 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 occurrence 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).

230 Statistical analyses

231 *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 ($|\text{loading}| > 0.4$).

	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 ($|\text{loading}| > 0.4$).

	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 ($|\text{loading}| > 0.4$).

	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

248 *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.

260 **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).
```

289 **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).
```

306 **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

318 **Results**319 Effect of positive handling at weaning and conditioning on piglets'
320 reaction to human presence (Isolation/Reunion tests)321 ***Piglets that were not handled at weaning express a similar behavioural proximity***
322 ***to a human after a positive conditioning as the positively handled ones.***

323

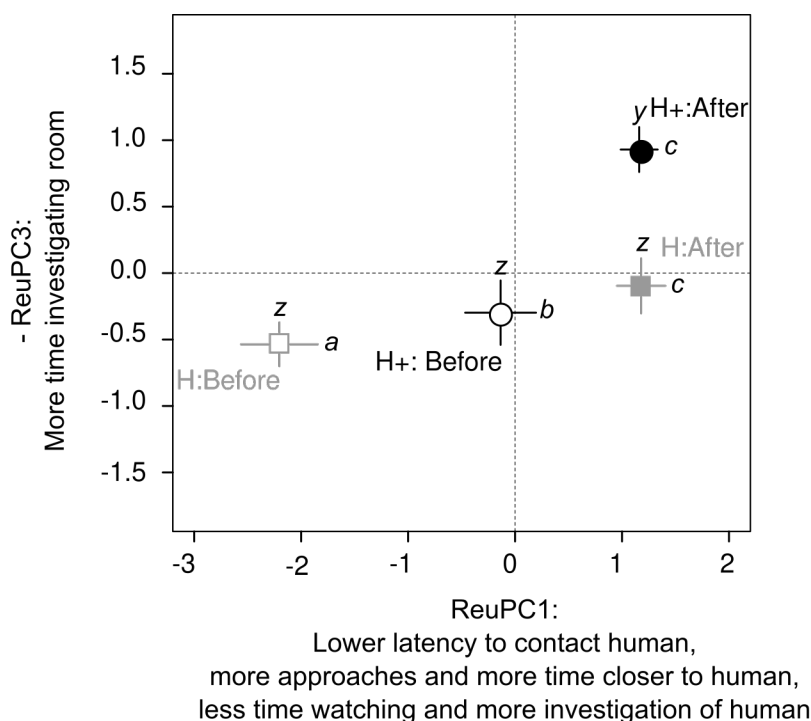


Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean \pm SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey squares 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)

324 The interaction between the treatment (positively handled piglets at weaning (H+) or not (H) and
325 the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3
326 ($\chi^2_1 = 28.0$, $p < 0.001$, and $\chi^2_1 = 3.7$, $p = 0.05$ respectively, figure 2) but not for ReuPC2 ($\chi^2_1 <$
327 0.001 , $p = 0.99$, supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was higher
328 after the conditioning than before (H: after – before, t.ratio = 12.1, $p < 0.001$, H+: after – before
329 t.ratio = 11.0, $p < 0.001$) and that before the conditioning, piglets that were positively handled at
330 weaning had significantly higher ReuPC1 than non handled piglets (Before, H – H+: t.ratio = -2.1, p
331 < 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).

344 ***Piglets positively handled at weaning produce shorter grunts even when no human***
 345 ***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 : $\chi^2_1 < 3.5$, $p > 0.06$, 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 ($\chi^2_1 > 6.6$, $p < 0.01$, table S1). During the reunion phase with the human,
 354 grunts were shorter (estimates of $\log(\text{duration})$ [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 ($\chi^2_1 = 5.5$, $p = 0.02$ and $\chi^2_1 = 4.9$, $p = 0.03$ respectively, table S2). Grunts produced by
 361 positively handled at weaning piglets were shorter (estimates of $\log(\text{duration})$ [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.**

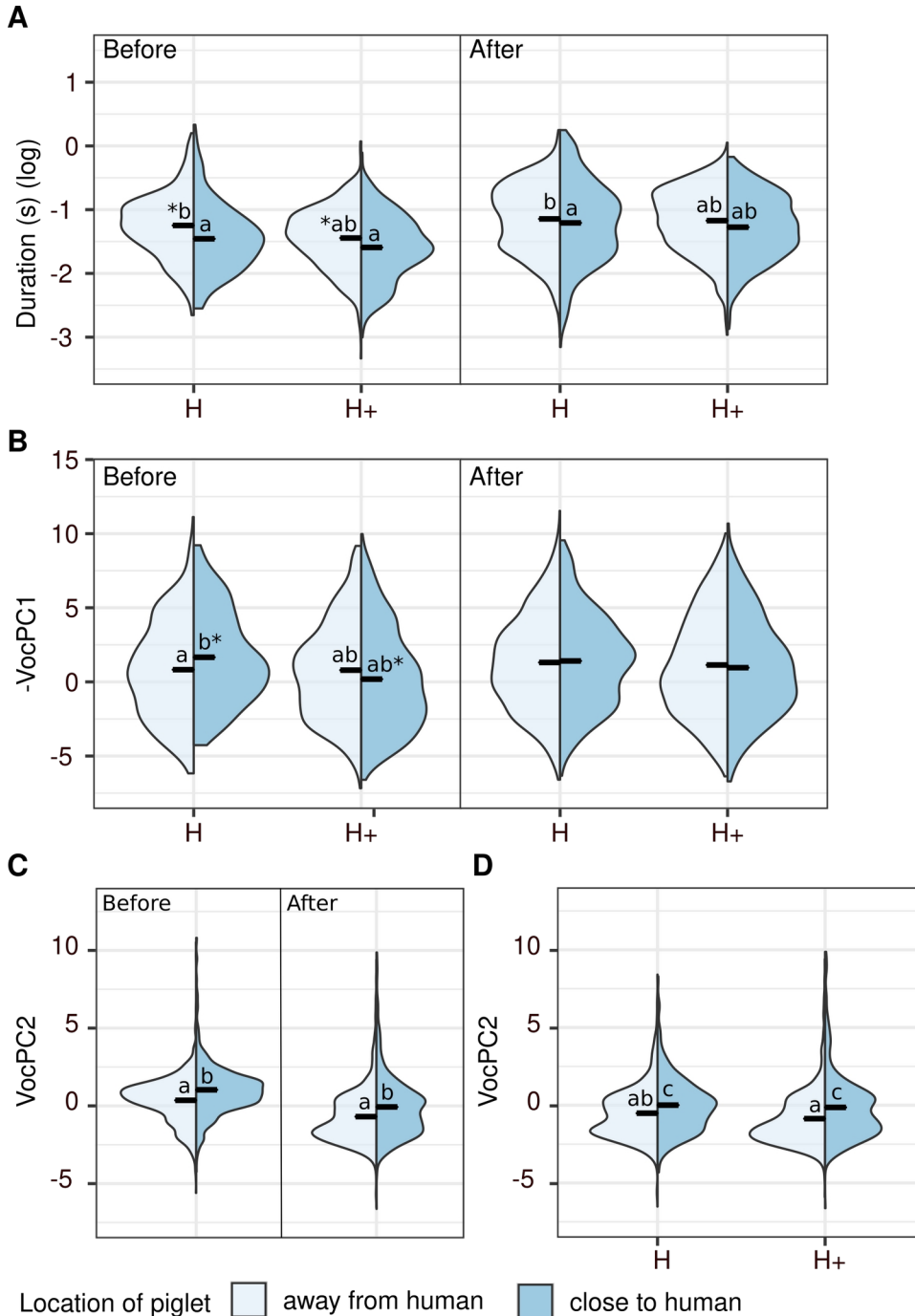


Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect of conditioning (before or after), treatment (H or H+) and location of the piglet relatively to the human (close: dark blue or away from them: light blue). Violin plots representing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

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

392 ***The conditioning increases behavioural proximity to the human in all piglets.***

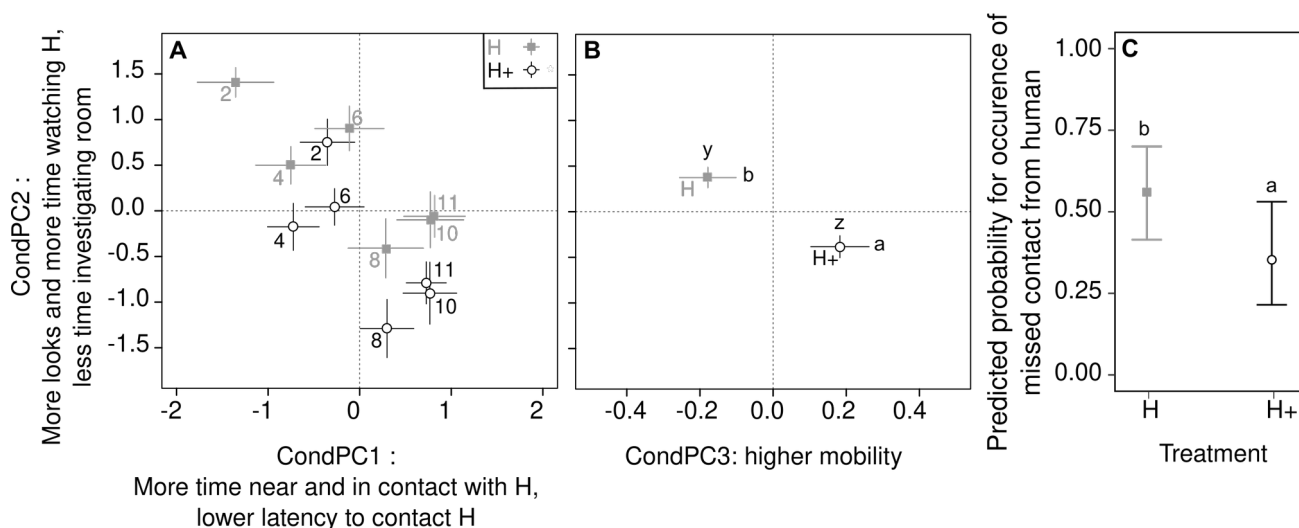


Figure 4: Behavioural variation of responses of piglets according to the sessions of additional positive contacts of the conditioning (A), and to the treatments (B, C). (A, B) Mean \pm SE per group, numbers in (A) refer to the trial number of the conditioning. Higher CondPC1 and lower CondPC2 over time (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 \pm 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).

393 No evidence of any effect of the interaction between the treatment [positively handled piglets
 394 before the conditioning (H+) or not (H)] and the trial number was found for all behavioural scores
 395 (CondPC1, CondPC2 and CondPC3, table 3). Independently from the treatment, the higher the trial
 396 number the higher CondPC1 ($\chi^2_1 = 59.3$, $p < 0.001$, slope estimate [95% confidence interval]: 0.20
 397 [0.15 : 0.25]) and the lower CondPC2 was ($\chi^2_1 = 48.6$, $p < 0.001$, slope estimate: -0.17 [-0.22 : -
 398 0.12]). According to the loadings, over the conditioning, piglets decreased the latency to contact the
 399 human, made more contacts, spent more time in the proximal area and in contact with the human
 400 (condPC1), decreased the number of looks to the human, spent less time watching the human and more
 401 time investigating the room (CondPC2) (figure 4A). Independently from the trial number, positively
 402 handled piglets had a lower CondPC2 and a higher CondPC3 than the non handled ones ($\chi^2_1 = 12.8$,
 403 $p < 0.001$ and $\chi^2_1 = 7.0$, $p = 0.008$ respectively), meaning that piglets that were positively handled at
 404 weaning expressed a fewer number of looks to the human, spent less time watching them and more
 405 time investigating the room (CondPC2) and crossed more virtual zone during the test (CondPC3)
 406 (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 ($\chi^2_1 = 9.57$, $p = 0.002$, figure 4C), with no
 408 interaction with the trial number ($\chi^2_1 = 0.22$, $p = 0.064$).

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

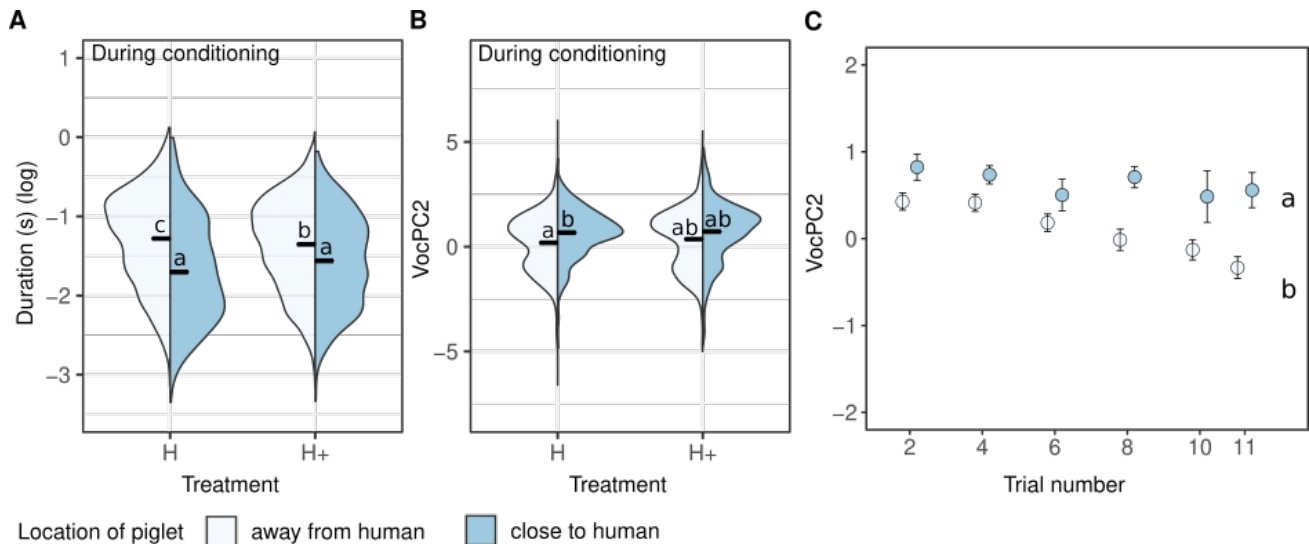


Figure 5: Vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human ('close': dark blue) or elsewhere in the room ('away' from the human: light blue) and treatment (H vs. H+ piglets) on grunt duration (A) and VocPC2 (B). (C) Mean \pm 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 ($\chi^2_1 < 0.18$, $p > 0.67$), 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 ($\chi^2_1 < 2.5$
 415 $p > 0.11$). Grunt duration decreased over time and independently from the treatment (trial
 416 number:replicate interaction, $\chi^2_1 < 5.3$ $p = 0.02$, 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: $\chi^2_1 = 15.8$ $p < 0.001$, 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, $\chi^2_1 = 3.97$ $p = 0.05$ and $\chi^2_1 = 6.1$ $p = 0.01$ 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, $\chi^2_1 = 7.6$ p = 0.005, pairwise comparisons away vs. close, in H:
 429 z.ratio = -4.9 p < 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

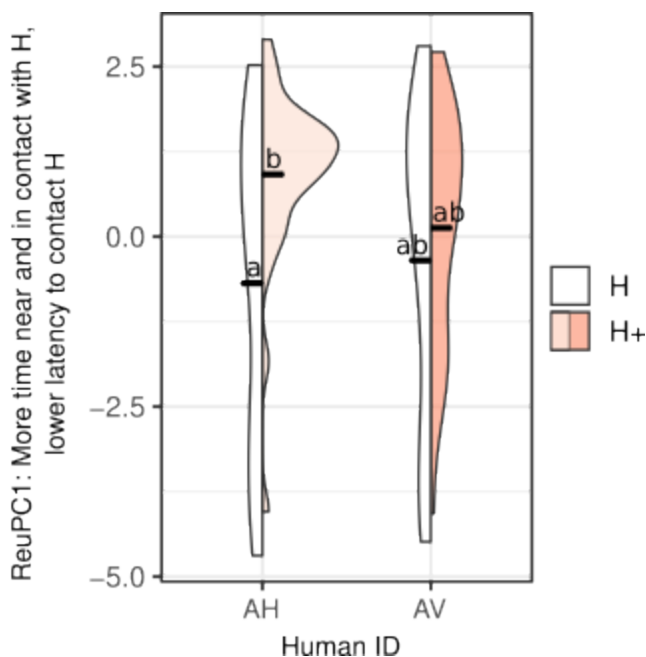


Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Values with no common letters differ significantly. Full statistical report is available as supplementary 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, $\chi^2_1 = 6.01$, p = 0.01) but
 439 not the others (ReuPC2 and ReuPC3 ($\chi^2_1 < 1.98$, 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 (VocCPI 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	Z-ratio = -2.82	P = 0.005
		AV – Trial number	-0.007	-0.031	0.016		

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 ($\chi^2_1 =$
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 ($\chi^2_1 = 8.0$, $p = 0.005$): 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).

466 **Discussion**

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.

483 Behavioural evidence of a rapid establishment of interest and 484 proximity toward a human providing additional positive contacts

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.

527 Links between vocal expression and positive HAR

528 *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.

554 *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.

584 ***Providing rewarding additional positive contacts triggers short and high pitched***
585 ***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.

630 Effect of human identity on piglets' perception: perspectives on 631 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.

678 **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.).

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689 **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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696 **non colored clean version**

697 **The use of pigs vocalisation structure to assess the quality of**
698 **human-pig relationship**

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708 **Key words**

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

711 **Abstract**

712 Studying human-animal interactions in domestic species and how they affect the establishment of a
713 positive Human-Animal Relationship (HAR) may help us improve animal welfare and better
714 understand the evolution of interspecific interactions associated with the domestication process.
715 Understanding and describing the quality of an HAR requires information on several aspects of the

716 animal biology and emotional states (social, spatial and postural behaviours, physiological and
717 cognitive states). Growing evidence shows that acoustic features of animal vocalisations may be
718 indicators of emotional states. Here, we tested the hypothesis that vocal structure may indicate the
719 quality of HAR. At weaning, 30 piglets were positively handled by an experimenter who talked to
720 and physically interacted with them three times a day, while 30 other piglets only received the
721 contact necessary for proper husbandry. After two weeks, we recorded the behaviours and
722 vocalisations produced in the presence of the static experimenter for five minutes. We repeated this
723 test two weeks later, after a conditioning period during which human presence with additional
724 positive contacts was used as a reward for all piglets. We hypothesized this conditioning period
725 would lead to a positive human-piglet relationship for all piglets. As expected, piglets that were
726 positively handled at weaning expressed a higher attraction toward the experimenter, and, after the
727 conditioning, piglets that were not positively handled at weaning expressed a similar level of
728 attraction than the positively handled ones. Piglets positively handled at weaning produced shorter
729 grunts than the other ones, regardless of the context of recording, which may indicate a more positive
730 affect. During reunions with the static experimenter, a more positive HAR was associated with a
731 decrease in vocal reactivity to human proximity. However, during reunions with the experimenter
732 providing additional positive contacts and over the conditioning, proximity to the human
733 systematically triggered shorter and higher pitched grunts, indicator of positive a emotional state.
734 Results first show that changes in vocal structure are consistent with indicators of positive states in
735 the presence of a human. Second, these changes are stronger when the human positively interact with
736 the piglets, supposedly emphasizing a higher positive arousal state during these interactions. We
737 show that vocalisation structure may be a promising indicator of the quality of human-pig
738 relationship.

739 **Introduction**

740 The process of domestication was conducted to shape physiology and morphology of domestic
741 animal species, but also their behaviour. It notably has shaped interspecific interactions between
742 human and non-human animals, by improving animals' capacity to use human signals to adapt their
743 behaviour both decreasing fearfulness toward humans and increasing attention toward humans
744 (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is
745 important for animal welfare. Therefore, studying human-animal interactions and their consequences
746 to understand the mechanisms of emergence and maintenance of a positive human-animal
747 relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three
748 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, Lanthony, et al., 2020) 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, some vocalisations type 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. Second, vocalisation structure is known to carry markers of the emotional states in several bird and mammal species (Briefer, 2012, 2020) and markers of emotional valence (positive versus negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Since positive or negative HAR is likely to affect the emotional state of animals, it is likely that it may be reflected in the structure of the produced vocalisations.

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;

784 Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related
785 to the valence or the arousal a situation may trigger for the animal. For example, the grunt, a contact
786 call, is used in various contexts and is now known to be a flexible call. Positive situations have been
787 associated with shorter grunts compared to negative ones (Briefer et al., 2019, 2022; Friel et al.,
788 2019), as well as higher formants (which are frequency peaks containing more energy than others)
789 and a lower fundamental frequency during positive situations (Briefer et al., 2019, 2022). Grunt
790 structure may also change according to the arousal of a negative situation: the higher the arousal in
791 the negative state the higher the frequency range and bandwidth (Linhart et al., 2015) and the longer
792 (Puppe et al., 2005) the grunts. Variation in grunt spectro-temporal structure in positive situations of
793 different arousal is still unknown.

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

805 **Methods**

806 Ethical note

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

813 Subjects and housing conditions

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

818 One piglet had to be excluded from our sample size to receive care/medication due to health issues
819 independent from the experiment. From weaning at 28 days of age, piglets from the same litter and
820 having similar weight (<1 kg difference) were housed by three in a 1.2 x 1.3m pen on plastic
821 duckboard. Wooden panels were used to visually isolate pens. One metal chain per pen was used for
822 enrichment. Food and water were available *ad libitum*. Artificial lights were turned on from 8:00 to
823 17:00 and temperature was maintained between 26 and 27 °C. The experiment was carried out in two
824 replicates and two identical rearing rooms were used (5 pens per room per replicate).

825 Treatment: positive handling at weaning

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

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

832 - **Positively handled piglets piglets (H+ piglets):** Experimental piglets from the 10 other rearing
833 pens, housed in another room, received the same daily care given by the same stockperson as for H
834 piglets. They additionally received repeated sessions of additional human contacts. Each pen of three
835 piglets received 29 sessions of 10 minutes, from day 28 (weaning) until day 39, occurring five days a
836 week. Three sessions per day were performed (except on the day of weaning during which only two
837 were done with a two-hour break in between). Each session took place in the rearing pen and the
838 order of the interventions in the pens was balanced across days. The handling procedure, using gentle
839 tactile contacts is described in supplementary material of Villain et al. (2020) and was similar to
840 Tallet et al. (2014). Two experimenters performed these sessions (both women, both between 1.70-
841 1.73 m tall, with a balanced number of pens attributed to each of them). The experimenters wore the
842 same blue overalls and green boots each time they interacted with the piglets. The experimenters
843 tried to imitate each others behaviours (remote video monitoring) to decrease variability.

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

848 Conditioning: sessions of additional positive contacts with 849 (un)familiar human

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

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

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

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

872 Standard Isolation/Reunion Tests with a static and silent human

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

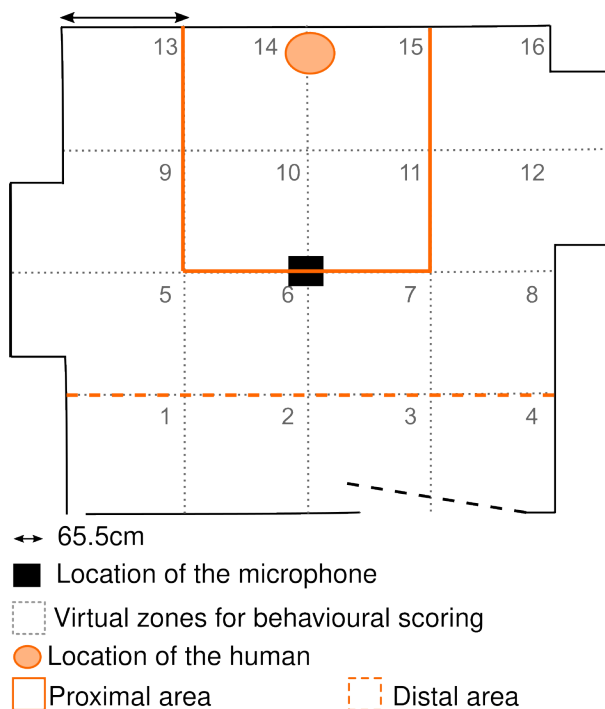


Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1, 2, 3, 4) were defined, using the location of the human as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

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

884 The behaviours scored during the reunion of the Isolation/Reunion test and the sessions of
 885 additional positive contacts of the conditioning are available in table 1. Every time the shoulders of
 886 the piglet crossed a zone, a zone change was scored. Looks and watching behaviours were scored as
 887 point events, all other behaviours were scored as state events. Behavioural scores were then
 888 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

889

890 Acoustic monitoring and analyses

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

903 A spectro-temporal analysis was performed with custom-written codes using the Seewave R
 904 package (Sueur et al., 2008) implemented in R (R Core Team, 2015). We first studied the spectral
 905 properties of the remaining background noise of the experimental room (electric noises and
 906 remaining low frequency noises from the rest of the building), using 20 examples of 0.5 second
 907 fragments. Since the first quartile (Q25) of the normalized spectrum of the background noise was
 908 250Hz and the grunts are low frequency vocalisations, we decided to remove all frequencies below
 909 200Hz in order to focus on the most relevant frequencies, using a 0.2-8 kHz bandpass filtering (‘fir’
 910 function). As a consequence, all results presented in this study are on a 0.2-8kHz frequency range,

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

926 Statistical analyses

927 ***Behavioural and vocal response scores***

928 All measures extracted from videos or sound analysis are named parameters throughout the text.
 929 The symmetrical distribution of parameters (behavioural on the one hand and acoustic on the other
 930 hand) was visually inspected, and linear transformations were computed when necessary to reach
 931 symmetrical distribution (see tables 2, 3, 4). When this criteria was reached, Principal Component
 932 Analyses (PCA, one for the behavioural analysis and one for the spectral acoustic analysis) were
 933 performed using several parameters to build scores ['dudi.pca' function from 'ade4' R package
 934 (Dray & Dufour, 2007) and 'inertia.dudi' function to extract the loadings]. These scores were then
 935 used as statistical variables. Indeed, PCAs are generally used to reduce the number of variables
 936 included in statistical models. It also generates quantifiable global descriptors of behaviours or
 937 acoustic structure, since correlated parameters usually load on the same PC (McGregor, 1992). All
 938 PCs having an eigenvalue above one were kept and constituted response scores of behavioural
 939 ('ReuPCs' and 'CondPCs' in table 2 and 3 respectively) and vocal ('VocPCs', table 4) parameters.
 940 Only the duration of grunts was kept separated from the spectral parameters to keep it as a temporal
 941 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 ($|\text{loading}| > 0.4$).

	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

942

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 ($|\text{loading}| > 0.4$).

	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

943

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 ($|\text{loading}| > 0.4$).

	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

944 *Statistical models*

945 All statistics were carried out on R (R Core Team, 2015). Linear mixed effect models [*lmer*
946 function, *lme4* R package (Bates et al., 2014)] were built when tested variables were linear
947 (behavioural and vocal scores, grunt duration) and one binomial generalized mixed effect model was
948 built for binary parameters (occurrence of missed contacts initiated by human during the
949 conditioning). The following subsections describe how models were built for each type of tests. In all
950 models described below, the identity of the replicate ('1' or '2') was used as an interacting fixed

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

956 **Isolation/Reunion tests**

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

```
964 Model_1 <- lmer (ReuPCs ~ Treatment*Time + Treatment*Replicate +
965 Treatment*HumanID + Time*Replicate + Time*HumanID + (1 | pigletID), data=
966 data_Behaviour_Reunion).
```

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

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

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

```
981 Model_3 <- lmer (Vocal response score ~ Treatment*Time*InProxArea +
982 Treatment*HumanID + InProxArea*HumanID + Treatment*Replicate +
983 InProxArea*Replicate + Time*Replicate + Time*HumanID + (1 | pigletID/Time), data
984 = data_Vocal_Reunion).
```

985 **Conditioning trials**

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

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

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

```
999 Model_5 <- lmer (VocPCs ~ Trial*Treatment*InProxArea+ Trial*HumanID +
1000 Trial*Replicate + Treatment*Replicate + Treatment*HumanID + HumanID*InProxArea +
1001 Replicate*InProxArea + (1+ Trial | pigletID), data= data_Vocal_Conditioning).
```

1002 **Model validation and statistical tests**

1003 All linear models were validated by visual inspection of the symmetrical and normal distribution of
1004 the residuals. Anovas (‘car’ R package (Fox & Weisberg, 2011)) were computed on models to test
1005 for significant effects of explanatory variables. Following the Anova, when interactions were found
1006 significant, post hoc test were run on model interactions, correcting for multiple testing with Tukey
1007 contrasts (‘emmeans’ or ‘lstrends’ functions from ‘emmeans’ R package (Lenth, 2016), for
1008 categorical or continuous variables respectively). Considering the conditioning time (before or after
1009 conditioning), when involved in a significant three-way interaction, this factor was fixed to allow
1010 pairwise comparison within each time period as it was not considered relevant to assess the effect of
1011 time only. Results of the Anova, model estimates and pairwise post hoc comparisons are reported in
1012 the supplementary material (tables S1 and S2 for tests, table S3 for model estimates).

1013

1014 **Results**1015 Effect of positive handling at weaning and conditioning on piglets'
1016 reaction to human presence (Isolation/Reunion tests)1017 ***Piglets that were not handled at weaning express a similar behavioural proximity***
1018 ***to a human after a positive conditioning as the positively handled ones.***

1019

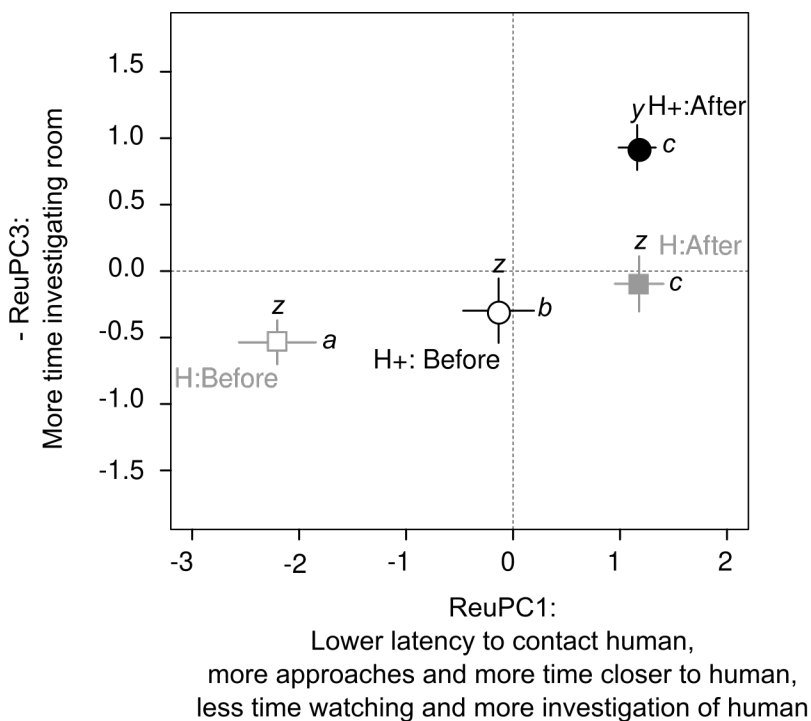


Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean \pm SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey squares 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)

1020 The interaction between the treatment (positively handled piglets at weaning (H+) or not (H) and
1021 the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3
1022 ($\chi^2_1 = 28.0$, $p < 0.001$, and $\chi^2_1 = 3.7$, $p = 0.05$ respectively, figure 2) but not for ReuPC2 ($\chi^2_1 <$
1023 0.001 , $p = 0.99$, supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was higher
1024 after the conditioning than before (H: after – before, t.ratio = 12.1, $p < 0.001$, H+: after – before
1025 t.ratio = 11.0, $p < 0.001$) and that before the conditioning, piglets that were positively handled at
1026 weaning had significantly higher ReuPC1 than non handled piglets (Before, H – H+: t.ratio = -2.1, p
1027 < 0.001), but not after (After, H – H+: t.ratio = 0.02, $p = 1.0$). According to the loadings, this means

1028 that piglets that were positively handled at weaning had a lower latency to contact the human,
 1029 approached them more often and spent more time close to and investigating the human (ReuPC1)
 1030 than non handled piglets, before the conditioning. This score increased after the conditioning and no
 1031 evidence of a difference between treatments after the conditioning was found (figure 2). Post hoc
 1032 tests on ReuPC3 showed a significant effect of the conditioning time only in piglets that were
 1033 positively handled at weaning (H+: after – before, t.ratio = 5.2, $p < 0.001$, H: after – before, t.ratio =
 1034 2.6, $p = 0.06$). No difference in ReuPC3 was found between treatments before the conditioning
 1035 (Before: H – H+, t.ratio = -0.75, $p = 0.87$), whereas positively handled piglets had a higher -ReuPC3
 1036 after the conditioning than before (After : H – H+, t.ratio = -3.2, $p = 0.009$). According to the
 1037 loadings, this means that after the conditioning, piglets that were positively handled at weaning
 1038 expressed more investigation of the room after the conditioning than non handled piglets. No
 1039 evidence of any effect on ReuPC2 was found (table S2).

1040 ***Piglets positively handled at weaning produce shorter grunts even when no human***
 1041 ***is present.***

1042 Using the isolation phase as a negative control we could compare the effect of the phase of the test
 1043 (Isolation vs. Reunion with the human), taking into account the conditioning time (before or after the
 1044 conditioning) and the treatment. No evidence of any effect of neither the three way interaction ($\chi^2_1 <$
 1045 0.62, $p > 0.43$) nor two way interactions of interest was found (treatment: phase, conditioning
 1046 time:phase, conditioning time: treatment interactions : $\chi^2_1 < 3.5$, $p > 0.06$, table S2) in any of the
 1047 scores.

1048 Regardless of the treatment, single effects of the phase of the test were significant for grunt
 1049 duration and all AcPCs ($\chi^2_1 > 6.6$, $p < 0.01$, table S1). During the reunion phase with the human,
 1050 grunts were shorter (estimates of log(duration)[95% CI] : -1.32[-1.37;-1.26] vs. -1.06[-1.12;-1.00]),
 1051 had a higher frequency range, higher bandwidth and a higher noise component (-VocPC1:
 1052 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[-
 1053 0.65;-0.28]) and their spectrum had a higher skewness and kurtosis (VocPC3: -0.25[-0.37;-0.14] vs. -
 1054 0.11[-0.23;0.01]), compared to the isolation phase.

1055 Regardless of the phase of the test, single effects of treatment were found for grunt duration and -
 1056 VocPC3 ($\chi^2_1 = 5.5$, $p = 0.02$ and $\chi^2_1 = 4.9$, $p = 0.03$ respectively, table S2). Grunts produced by
 1057 positively handled at weaning piglets were shorter (estimates of log(duration)[95% CI]: -1.25[-1.32;-
 1058 1.19] vs. -1.12[-1.2;-1.1], table S3), and differed in -VocPC3 scores, describing the shape of the

1059 frequency spectrum (estimates of $-VocPC3$ [95% CI]: $-0.29[-0.43;-0.14]$ vs. $-0.07[-0.22;0.08]$, table
 1060 S3), than grunts produced by non handled piglets.

1061 **Positive handling and conditioning affect vocal reactivity to human proximity.**

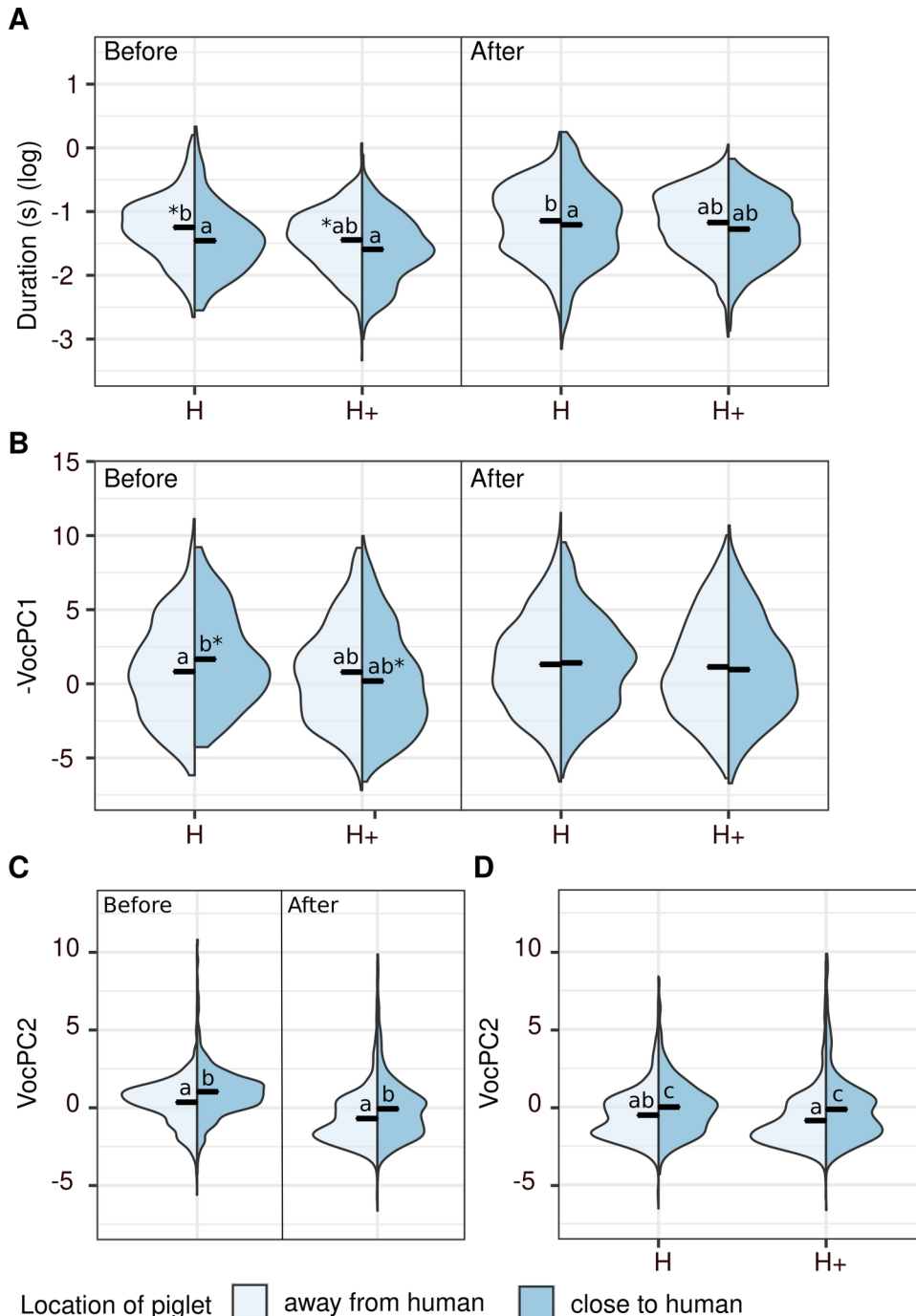


Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect of conditioning (before or after), treatment (H or H+) and location of the piglet relatively to the human (close: dark blue or away from them: light blue). Violin plots representing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

1062 During the five-minute reunion, the piglet was scored either as close to the human or away from
1063 them. The three way interaction of the conditioning time, the treatment and the location was
1064 significant for grunt duration, -VocPC1 and VocPC3 ($\chi^2_1 > 4.9$, $p < 0.03$). Post hoc tests revealed
1065 that grunts produced closer to the human were shorter than the ones produced further away, but only
1066 in piglets that were not positively handled at weaning, effect being stronger before the conditioning
1067 than after it (H piglets: away – close, z.ratio = 6.3, $p < 0.001$ before and z.ratio = 4.1 $p < 0.001$ after
1068 the conditioning; H+ piglets: away – close z.ratio < 1.98 $p > 0.19$, figure 3A). -VocPC1 was higher,
1069 i.e. grunts had a higher frequency range, bandwidth and were noisier when produced closer to the
1070 human than further away, but only in non handled piglets and before the conditioning (H piglets:
1071 away – close, z.ratio = -3.34, $p = 0.005$ before and z.ratio = -1.23 $p = 0.61$ after the conditioning; H+
1072 piglets: away – close, z.ratio < 0.36 $p > 0.21$, figure 3B). For VocPC2, the three way interaction did
1073 not reach significance ($\chi^2_1 = 3.3$, $p = 0.07$), so only subsequent two way interactions were
1074 considered (post hoc tests on the three way interaction can be found in supplementary, tables S1 to
1075 S3). For VocPC2, significant two way interactions were found between the conditioning time and the
1076 location ($\chi^2_1 = 10.3$, $p = 0.001$) on the one hand, and between the location and the treatment ($\chi^2_1 =$
1077 4.2, $p = 0.04$) on the other hand. Post hoc tests revealed that grunts produced closer to the human had
1078 a higher VocPC2, meaning they had a higher pitch, effect being stronger before the conditioning than
1079 after (before: away – close, z.ratio = -6.12, $p < 0.001$; after: away – close, z.ratio = -2.88, $p = 0.004$,
1080 figure 3C). The increase in VocPC2 with the location was greater for non handled piglets than
1081 positively handled piglets (H piglets: away – close, z.ratio = -5.54, $p < 0.001$; H+ piglets: away –
1082 close, z.ratio = -3.82, $p = 0.001$, figure 3D). The last two-way interaction of interest between the
1083 conditioning time and the treatment did not reach significant level ($\chi^2_1 = 0.80$, $p = 0.37$). For
1084 VocPC3, post hoc tests did not reach significant levels ($|z.ratio| < 2.3$ $p > 0.09$ for any comparison) .

1085

1086 Emergence of positive perception of human (effect of additional
 1087 positive contacts sessions over the conditioning)

1088 ***The conditioning increases behavioural proximity to the human in all piglets.***

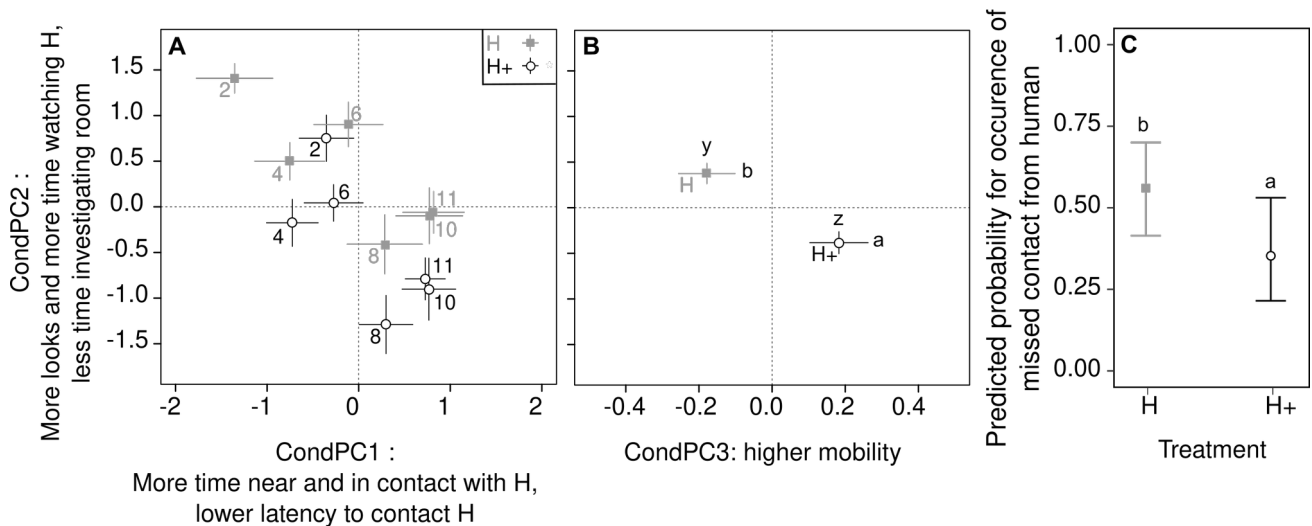


Figure 4: Behavioural variation of responses of piglets according to the sessions of additional positive contacts of the conditioning (A), and to the treatments (B, C). (A, B) Mean \pm SE per group, numbers in (A) refer to the trial number of the conditioning. Higher CondPC1 and lower CondPC2 over time (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 \pm 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).

1089 No evidence of any effect of the interaction between the treatment [positively handled piglets
 1090 before the conditioning (H+) or not (H)] and the trial number was found for all behavioural scores
 1091 (CondPC1, CondPC2 and CondPC3, table 3). Independently from the treatment, the higher the trial
 1092 number the higher CondPC1 ($\chi^2_1 = 59.3$, $p < 0.001$, slope estimate [95% confidence interval]: 0.20
 1093 [0.15 : 0.25]) and the lower CondPC2 was ($\chi^2_1 = 48.6$, $p < 0.001$, slope estimate: -0.17 [-0.22 : -
 1094 0.12]). According to the loadings, over the conditioning, piglets decreased the latency to contact the
 1095 human, made more contacts, spent more time in the proximal area and in contact with the human
 1096 (condPC1), decreased the number of looks to the human, spent less watching the human and more
 1097 time investigating the room (CondPC2) (figure 4A). Independently from the trial number, positively
 1098 handled piglets had a lower CondPC2 and a higher CondPC3 than the non handled ones ($\chi^2_1 = 12.8$,
 1099 $p < 0.001$ and $\chi^2_1 = 7.0$, $p = 0.008$ respectively), meaning that piglets that were positively handled at
 1100 weaning expressed a fewer number of looks to the human, spent less time watching them and more
 1101 time investigating the room (CondPC2) and crossed more virtual zone during the test (CondPC3)
 1102 (figure 4B). The probability of having at least one missed contact by the human during a session was

1103 lower for positively handled piglets than non handled ones ($\chi^2_1 = 9.57$, $p = 0.002$, figure 4C), with no
 1104 interaction with the trial number ($\chi^2_1 = 0.22$, $p = 0.064$).

1105 ***Additional positive contacts trigger shorter and higher pitch grunts in all piglets.***

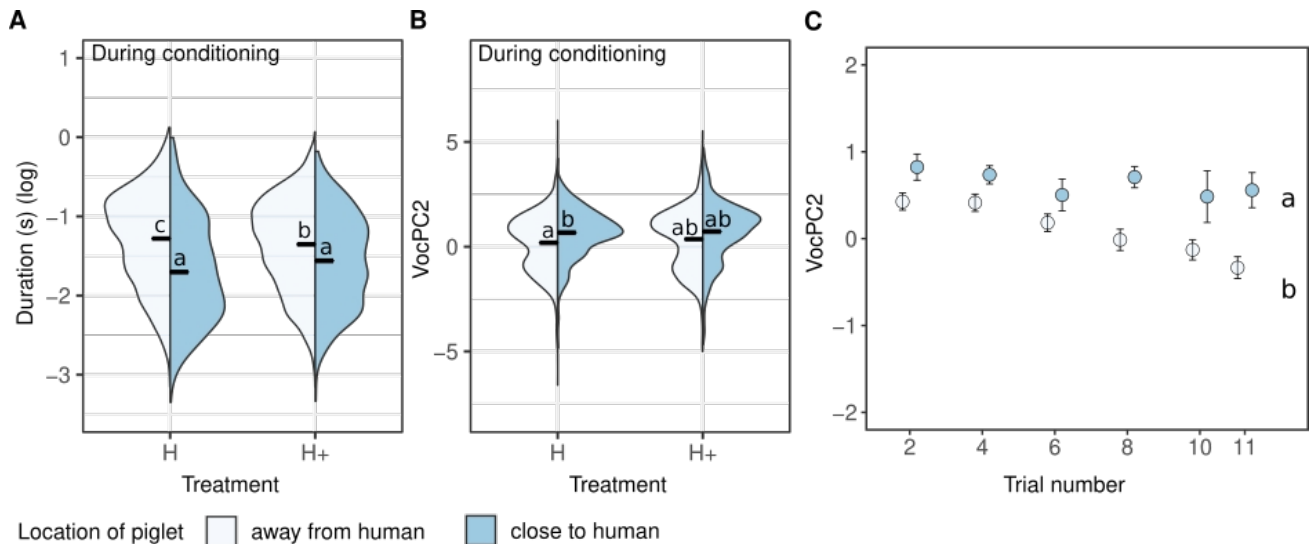


Figure 5: Vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human ('close': dark blue) or elsewhere in the room ('away' from the human: light blue) and treatment (H vs. H+ piglets) on grunt duration (A) and VocPC2 (B). (C) Mean \pm 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).

1106

1107 During the sessions of additional positive contacts of the conditioning, the three-way interaction
 1108 between the trial number, the treatment and the location was not significant for any of the vocal
 1109 scores ($\chi^2_1 < 0.18$, $p > 0.67$), allowing the analysis of the two way interactions of interest. The
 1110 interaction between treatment and the trial number was not significant for all vocal scores ($\chi^2_1 < 2.5$
 1111 $p > 0.11$). Grunt duration decreased over time and independently from the treatment (trial
 1112 number:replicate interaction, $\chi^2_1 < 5.3$ $p = 0.02$, slope estimate $-0.03[-0.04;-0.01]$ for the lower slope,
 1113 table S1 and S3). However, independently from the trial number, grunt duration was lower when
 1114 piglets were located close to the human and this effect was stronger in non handled piglets than
 1115 positively handled piglets (treatment:location interaction: $\chi^2_1 = 15.8$ $p < 0.001$, away vs.. close, H
 1116 piglets: z.ratio = 10.2 $p < 0.001$, H+ piglets: z.ratio = 6.86 $p < 0.001$, figure 5A). -VocPC1 and
 1117 VocPC2 decreased over time but remained higher when piglets were located close to the human (trial
 1118 number: location interaction, $\chi^2_1 = 3.97$ $p = 0.05$ and $\chi^2_1 = 6.1$ $p = 0.01$ respectively for -VocPC1 and
 1119 VocPC2). According to the loadings, this means that the frequency range, bandwidth and noisiness
 1120 of grunts (-VocPC1) as well as the pitch (VocPC2) decreased over the conditioning when piglets
 1121 were located away from the human but remained high when piglets were close (slope comparison

1122 away – close, -VocPC1 : z.ratio = -1.80 p = 0.07, VocPC2 : z.ratio = -2.34 p = 0.02, figure 5C).
 1123 Additionally, VocPC2 was higher when piglets were close to the human in non handled piglets
 1124 (treatment:location interaction, $\chi^2_1 = 7.6$ p = 0.005, pairwise comparisons away vs. close, in H:
 1125 z.ratio = -4.9 p < 0.001 and in H+: z.ratio = -2.0 p = 0.21), meaning that non handled piglets
 1126 produced higher pitched grunts when closer to the human (figure 5B).

1127 Impact of human identity on piglets behaviour and grunt structure

1128

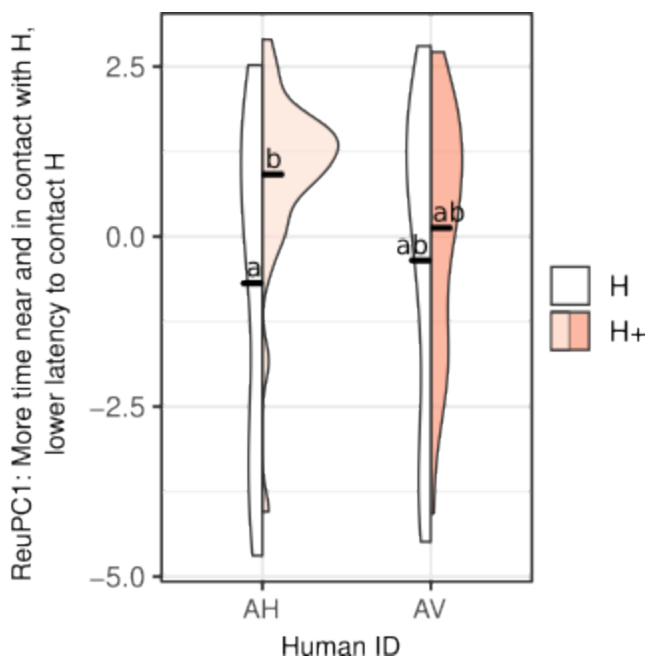


Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Values with no common letters differ significantly. Full statistical report is available as supplementary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

1129 Since half of the piglets had been assigned to one human experimenter and the other half to another
 1130 one, the identity of the human was included in the model. This allowed to test interactions between
 1131 the identity of the human and the treatment of positive handling at weaning on the one hand and the
 1132 conditioning time on the other hand.

1133 During the reunions of the Isolation/Reunion test, the interaction between treatment and human
 1134 identity was significant for the first behavioural proximity score (ReuPC1, $\chi^2_1 = 6.01$, p = 0.01) but
 1135 not the others (ReuPC2 and ReuPC3 ($\chi^2_1 < 1.98$, p > 0.16, table S1). The effect of treatment on
 1136 ReuPC1 was higher when piglets were handled by the human 'AH' (H vs. H+, AH: t.ratio = -4.77, p
 1137 < 0.001, figure 6). When the human 'AV' handled the piglets, for which ReuPC1 scores exhibited
 1138 intermediate values, treatment was not significant (AV, H vs. H+: t.ratio = -1.33, p = 0.56). These

1139 interacting effects of the human identity and treatment on behaviour were not found when
1140 considering the reunions of the conditioning ($\chi^2_1 < 1.32$, $p > 0.25$ for all CondPCs, table S1).

1141 Interactions between the human identity and conditioning time were not significant, neither
1142 considering the reunions of the Isolation/Reunion test (ReuPCs, $\chi^2_1 < 0.642$, $p > 0.42$, tables S1),
1143 neither the trial number during the session of additional positive contacts of the conditioning
1144 (CondPCs, $\chi^2_1 < 0.11$ $p > 0.74$, table S1).

Table 5: Significant effects of human identity on vocal response score (VocCPI 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	Z-ratio = -2.82	P = 0.005
		AV – Trial number	-0.007	-0.031	0.016		

1145

1146 Considering the vocal scores, no effect of human identity was found on VocPC1 during the
1147 Isolation/Reunion tests but -VocPC1 was higher when the human 'AH' was in the room during the
1148 reunion periods of the conditioning (table 5), meaning the frequency range and the bandwidth of the
1149 grunt were higher when the human 'AH' interacted with the piglet compared to the human 'AV'.
1150 VocPC2 was higher when the human 'AH' was in the room during the Isolation/Reunion tests (table
1151 5), meaning that the pitch of grunts was higher and this effect was also found during the sessions of

1152 additional positive contacts of the conditioning in interaction with the location of the piglet ($\chi^2_1 =$
1153 11.9, $p = 0.001$): VocPC2 increased when piglets were located close to the human but this increase
1154 was significant only for the human 'AV' and not for 'AH' (table 5). VocPC3 was not different
1155 between humans during the reunions of the Isolation/Reunion tests but, over the conditioning,
1156 VocPC3 changed differently when piglets were handled by the human 'AH' or 'AV', as showed by
1157 the significant interaction between trial number and human identity ($\chi^2_1 = 8.0$, $p = 0.005$): the
1158 skewness and kurtosis of grunts decreased over the conditioning when 'AH' was interacting with the
1159 piglets, but not 'AV' (see slope estimates, table 5). No evidence of any effect of human identity was
1160 found on grunt duration neither during the Isolation/Reunion tests nor during the sessions of
1161 additional positive contacts of the conditioning (table S1).

1162 **Discussion**

1163 In this study, familiarity to a human and human-animal interactions were experimentally modified
1164 in weaned piglets to study the establishment of a positive HAR and test whether grunt structure could
1165 reflect a positive HAR. A positive conditioning paradigm, using additional positive contacts from a
1166 human as a reward, allowed to compare the behavioural changes over time in piglets previously
1167 positively handled at weaning or not. Two types of sessions were studied: a standard
1168 isolation/reunion tests with the human, carried out before and after conditioning, during which the
1169 human remained silent and did not interact with the piglet, and sessions of the conditioning, during
1170 which the human interacted with the piglets, providing additional positive contacts, as long as the
1171 piglets stayed close to the seated human. Behavioural data were collected to describe the positive
1172 HAR. Grunts produced during the tests and sessions were collected and their spectro-temporal
1173 structure confronted to the behavioural data, with the hypothesis that vocalisation structure may
1174 reflect the quality of HAR, though vocal markers of positive emotions. Firstly, the discussion will
1175 focus on the behavioural validation of the establishment of a positive HAR. Secondly, behavioural
1176 and vocal expression will be confronted to discuss grunt spectro-temporal structure as indicator of
1177 the quality of HAR. Last, we will discuss perspectives regarding the effect of human identity on the
1178 establishment of a positive HAR.

1179 Behavioural evidence of a rapid establishment of interest and 1180 proximity toward a human providing additional positive contacts

1181 The standard reunion test with the human before the conditioning showed first that the treatment of
1182 positive handling at weaning succeeded in creating two different levels of human-piglet relationship

1183 (H and H+), as positively handled piglets expressed a higher attraction toward the human than non
1184 handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al.,
1185 2020). Second, this test showed that the conditioning increased the behavioural proximity toward the
1186 human of both positively handled and non handled piglets so that non handled piglets expressed a
1187 similar attraction toward the human as positively handled piglets. These results are in line with the
1188 behavioural results of the sessions of additional positive contacts. The analysis of piglets' behaviour
1189 every second sessions of the conditioning showed that, although positively handled and non handled
1190 piglets started with different degree of proximity toward the human (trials 2 and 4, CondPC1), then,
1191 over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human
1192 (CondPC1) and avoided less the human when the latter attempted to interact with them. So it seems
1193 that the conditioning process allowed non handled piglets to compensate the lack of positive
1194 handling before the conditioning and develop a similar proximity toward the human. Two minute
1195 daily sessions of additional positive contacts changed positively the perception of the human for the
1196 piglets, and thus their willingness to interact with them. Since no evidence of any interaction
1197 between time and treatment was found, no conclusion on differential developmental trajectories
1198 between treatments can be drawn, but a parallel development of the human-piglet relationship in
1199 both groups, when considering the proximity.

1200 Beside behavioural proximity, piglets that were positively handled at weaning expressed more
1201 exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also
1202 observed during the sessions of additional positive contacts of the conditioning: positive handled
1203 piglets started with a higher score associated with investigation than non handled piglets (CondPC2)
1204 and it held over the conditioning. Piglets that were positively handled at weaning also expressed a
1205 higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an
1206 expression of natural foraging and disinterest from human contact, which may be a sign of positive
1207 welfare (Weerd & Day, 2009). In addition, this could also be interpreted in terms of attachment to
1208 the human. Indeed, attachment to a human may facilitate exploration of novel environments or
1209 objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may
1210 provide an environment secure enough for the piglets to explore their environment in the presence of
1211 the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al.,
1212 2009).

1213 Overall, the behavioural monitoring showed that two minute sessions of positive additional
1214 contacts per day are sufficient to increase proximity to a human to similar levels as when piglets were

1215 previously familiarised for 2 weeks, even when piglets experienced social isolation. But it did not
1216 allow the non handled piglets to express natural exploratory behaviours as the positively handled
1217 piglets. We hypothesize a sequential establishment of a positive HAR over time: firstly with a
1218 decrease of attentive state and an increase in proximity and accepted contacts, and secondly with a
1219 disinterest of human contacts and the expression of natural foraging behaviour. The latter may
1220 require a higher exposure time.

1221 In the next paragraph we discuss to what extent changes in grunt spectro-temporal structure may
1222 reflect behavioural changes linked to the positive HAR over time.

1223 Links between vocal expression and positive HAR

1224 *A positive HAR is reflected by shorter grunts in presence and absence of a human*

1225 The social isolation phase of the Isolation /Reunion test, before any human entered the room, was
1226 associated with longer, lower pitched grunts with a downshifted frequency spectrum, whereas the
1227 reunion with a static human changed grunts structure to shorter, higher pitched with an upshifted
1228 frequency spectrum and this was observed in both handled and non handled piglets (H or H+) as well
1229 as before and after the conditioning. In terms of emotional indicators, similar changes in acoustic
1230 features of grunts were found in studies focusing on vocal markers of valence in pigs (Briefer et al.,
1231 2019, 2022; Friel et al., 2019; Villain, Hazard, et al., 2020), meaning that the reunion with a human,
1232 after a period of social isolation would be perceived as positive. However, this modulation of grunt
1233 structure was observed regardless of piglet experience with the human. It is possible that the reunion
1234 with an either neutral or familiar human, releasing piglets from total isolation could be perceived as
1235 positive by the piglets, as suggested in previous studies (Villain, Lanthony, et al., 2020).

1236 In addition, and surprisingly, positively handled piglets produced shorter grunts than non handled
1237 piglets regardless of human presence. This was previously shown in another context (anticipation of
1238 (pseudo)social events independently from the type of partner) in the same groups of piglets (Villain,
1239 Hazard, et al., 2020). This may show that the period of positive handling at weaning modulated vocal
1240 expression in the long term, as this result was found both before and after the conditioning. On the
1241 one hand, a positive HAR establishes through successive positive experiences (Rault et al. 2020)
1242 and, on the other hand, HAR may have long term effects on behavioural expressions, as suggested by
1243 Brajon et al. (2015) using cognitive bias tests. We can thus hypothesize this may also be reflected in
1244 the way piglets vocalise, in general. In that case, we may have evidence of expression of another

1245 category of affect, moods, and not only emotional expression. Indeed, as suggested by Schnall
1246 (2010), although emotions are short-term affects triggered by an external stimulus, moods, on the
1247 other hand, may be experienced on a longer term and may not be attributable to a specific stimulus.
1248 Although emotions and moods do not rely on the same time scale, they may interact with one
1249 another, and more studies are needed to understand their effects on vocal expression.

1250 *A positive HAR affects vocal reactivity toward a static human*

1251 In a previous study, we showed that pigs vocalizing close to a human that previously had provided
1252 repetitive additional positive contacts produced shorter and higher pitch grunts, compared to when
1253 vocalizing away from the human (Villain, Lanthony, et al., 2020). Using the same type of test with
1254 positively handled at weaning and non handled piglets, before or after conditioning sessions with
1255 positive interactions, we can test the effect of positive handling on this modulation of grunt structure.
1256 Similarly to the previous study, during the standard reunion test (no contact from the human), piglets
1257 produced shorter and higher pitched grunts with an upshifted frequency spectrum when close to the
1258 human. It has to be noted that this effect was 1) stronger in previously non handled piglets than
1259 positively handled at weaning piglets and 2) stronger before the conditioning than after. In other
1260 words, the more familiar with the human associated with positive handling, the less reactive to
1261 human proximity.

1262 These results may be interpreted according to the behavioural results we described earlier (fig. 2).
1263 We described that the proximity to the human was first increasing at the beginning of positive
1264 handling experiences (see H piglets, before vs. after conditioning) before reaching a maximum (see
1265 H vs. H+ piglets after conditioning) and that the most familiar piglets showed more exploratory
1266 behaviours (H+ after conditioning). The acoustic results during the standard reunion mirror the
1267 behavioural results from the same test. The least familiar piglets would vocally express the
1268 exploration of a neutral and static human and, as the familiarity with the human increases, the human
1269 may become part of their environment, explaining the lack of vocal reactivity when close to the static
1270 human.

1271 In addition, we may also be facing ceiling effects in terms of vocal flexibility, which could also
1272 partly explain these results. We showed that positively handled piglets generally produce shorter
1273 grunts than non handled piglets, and that the shape of the frequency spectrum of these grunts was
1274 different. So the structure of their calls, in general is different. According to the source-filter theory
1275 of vocal production, vocal flexibility is constrained by the dimensions and functioning of the vocal

1276 apparatus (lung capacity, characteristics of the vocal folds, length and shape of the vocal tract, see
1277 (Taylor & Reby, 2010) and (Titze & Martin, 1998)). It is possible that the positive HAR developed
1278 by the positively handled piglets may have change their grunts structure to an extent that vocal
1279 flexibility is no longer quantifiable in the experimental design of this study.

1280 ***Providing rewarding additional positive contacts triggers short and high pitched***
1281 ***grunts***

1282 Contrary to the standard reunions with a static human, the human actively interacted with the
1283 piglets during the sessions of the conditioning, providing contacts and producing speech as long as
1284 the piglets remained close to the human. During these sessions and contrary to the standard reunions,
1285 grunts produced close to the human were shorter and higher pitched, regardless of the trial number of
1286 the conditioning and treatment. Although these effects were stronger in non handled piglets than
1287 positively handled piglets, they remained over time. We describe here two types of vocal reaction to
1288 human proximity, depending on the human behaviour. On the one hand, time decreased vocal
1289 reactivity to human proximity during a standard reunion with a static human. On the other hand, no
1290 evidence of a decrease in vocal reactivity to human proximity was found during sessions of
1291 additional positive contacts. This would mean that positive interactions with piglets consistently
1292 triggers the production of shorter and higher pitch grunts. These changes may be explained by the
1293 expression of a higher arousal state experienced by the piglets while being positively handled.
1294 Indeed, in the context of these sessions, the piglet could choose to approach and stay close to the
1295 human, which will provide positive contacts systematically. So the piglet may anticipate to receive
1296 positive contact and systematically being rewarded. When close to the human, observed changes in
1297 frequency distribution of grunts (increased pitch and upshifted frequency spectrum) are known to be
1298 markers of arousal (in the negative state in multiple mamalian species (Briefer, 2012, 2020) and pigs
1299 (Linhart et al., 2015)). In addition, these spectral changes were also associated with shorter grunts.
1300 Although the duration of grunts is associated with the valence of a situation, the duration may also be
1301 an indicator of positive arousal. This hypothesis has to be taken precociously since no additional
1302 control of arousal could be done in the present study.

1303 This working hypothesis may explain the decrease in vocal reactivity to human proximity observed
1304 during the standard reunion test as the HAR becomes more positive. Indeed, before the sessions of
1305 the conditioning, positively handled piglets were habituated to a human interacting positively when
1306 present whereas non handled piglets were not, hence, during the first standard reunion test, when the
1307 human is present but do not interact with the piglet, positively handled and non handled piglets may

1308 have diverging expectations regarding the presence of the static and silent human. As positively
1309 handled piglets received positive contacts every time they were in the presence of the human, they
1310 may have expected positive contacts when approaching and experienced an absence of reward during
1311 the test. This has already been hypothesised in piglets deprived from human voice during interactions
1312 after a period of habituation to it (Bensoussan et al. 2020). On the contrary, piglets that were not
1313 positively handled at weaning never experienced additional positive contacts and being close to a
1314 human, having the possibility to investigate them may be some kind of reward after the period of
1315 total isolation. After the conditioning, piglets from both treatments were conditioned to receive
1316 additional positive contacts and both groups had experienced a first standard reunion test, so they
1317 may both experience an absence of reward during the test, which may explain a lower reaction to
1318 human proximity, and thus fewer changes on grunt spectro-temporal features.

1319 Last, we can raise the question whether changes in grunt structure in reaction to rewarding positive
1320 contacts may also be associated with a specific human-pig communication. In other domestic
1321 species, owner directed vocalisations has been shown (in cats, reviewed in (Turner, 2017); in dogs
1322 (Gaunet et al., 2022)). In addition, studies have found similar socio-communicative behaviours
1323 toward a human in socialized pigs and dogs (Gerencsér et al., 2019). Hence, we may profit from
1324 testing the existence of human directed vocalisations in pigs, as consequences of their socio
1325 communicative abilities.

1326 Effect of human identity on piglets' perception: perspectives on 1327 HAR

1328 We found that the identity of the human had effects on behavioural and vocal response scores.
1329 Piglets that were handled by the human 'AH' had higher values of behavioural proximity (ReuPC1)
1330 than piglets handled by the human 'AV' during reunion test after a period of isolation. This effect
1331 was not found during conditioning sessions. The effect of the human did not interact with the
1332 conditioning time, leading to the conclusion that the difference between the two experimenters may
1333 have established during the period of positive handling at weaning, prior to the conditioning.
1334 Additionally, when the human 'AH' was in the room, piglets produced grunts with a more upshifted
1335 frequency spectrum and a higher pitch than when the human 'AV' was in the room. If upshifted
1336 grunts may be a indicator of positive higher arousal, then we may conclude that 'AH' was more
1337 likely to trigger higher positive states than 'AV'. Interestingly, the human identity and the spatial
1338 proximity had different effects on piglets grunts during sessions of additional positive contacts but
1339 not when the human was static during the standard reunion test. Hence, it is possible that the way one

1340 human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering
1341 positive emotions and thus modifications of grunt structure. Several evidence exists in the literature
1342 that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al.,
1343 2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We
1344 may question the efficiency of different human features to generate a positive HAR. In our study,
1345 both humans that interacted with the piglets wear exactly the same clothes and standardized their
1346 tactile interactions toward the piglets before starting the study, and agreed on the rhythm and types of
1347 sounds (words, intonation) to use, to minimise generating variability although no systematic controls
1348 of the human behaviour or spectral feature of voices were performed here. It thus remains unclear
1349 whether experimenters interacted differently or if they were initially perceived differently by piglets.
1350 Our results show that the identity of the human may modulate piglet proximity and vocal behaviour
1351 but the design of this experiment does not allow to find the causes of these observations (behaviour,
1352 voice characteristics, or even odour profile). Thus, more studies of human features that are most
1353 likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In
1354 addition, studying human-piglet relationship in a more systematic way, as in other domestic species,
1355 for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech
1356 (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies
1357 of interspecific relationships. However, the influence of human identity did not modify the general
1358 outcomes of our study, but only decreased some effects, suggesting that this variability does not
1359 modify the main results, but should be considered in future studies.

1360

1361 To conclude, we showed that degrees of familiarity toward a human could be reflected in the way
1362 piglets vocalise in their presence, and out of it. We also showed that the spatial proximity toward a
1363 human providing additional care could change the acoustic structure of piglet grunts. These changes
1364 are likely to be linked to positive and more intense emotional states than when piglets are further
1365 away from the human. However, it is still unclear whether the changes in grunt structure could also
1366 be linked to human-animal communication and more studies are needed to determine it. We did also
1367 show that the identity of the human may be of importance, and may generate vocal changes during
1368 additional positive contacts that were not associated with changes in behaviour of the human. More
1369 systematic studies of human behaviour along with pig behaviour during the human-animal
1370 interactions would be needed to have a better understanding of the evolution of HAR, especially
1371 interactive interspecific communication as well as providing new procedures to promote positive

1372 welfare. We suggest that analysing vocalisations structure may be a good tool to assess the quality of
1373 human-pig relationship and help monitor the establishment of a positive HAR.

1374 **Authors contributions**

1375 Conceived and designed the experiment (A.V., C.T., C.N.). Performed the experiment (A.V., C.G.).
1376 Collection and processing of the acoustic and behavioural data (A.V., C.G.). Statistical analyses
1377 (A.V.). Contributed to the writing of the manuscript (A.V., C.T., C.N.).

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1385 **Data availability**

1386 The datasets used for the study are available at (Villain et al., 2022). The folder contains all datasets
1387 and a readme to match the type of analysis to the proper dataset. We have made sure to report in the
1388 main text of the article which R libraries and which functions in these libraries we used. All formulas
1389 of the statistical models are explicit in the text to facilitate transfer of information and replicate the
1390 analysis. All libraries are open source as well.

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