

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

## 3 **Authors**

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## 12 **Key words**

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

## 15 **Abstract**

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

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 5 min. We repeated this test  
27 two weeks later, after a conditioning period during which human presence with additional positive  
28 contacts was used as a reward for all piglets. We hypothesized this conditioning period would lead to  
29 a positive human-piglet relationship for all piglets. As expected, piglets that were positively handled  
30 at weaning expressed a higher attraction toward the experimenter, and, after the conditioning, piglets  
31 that were not positively handled at weaning expressed a similar level of attraction than the positively  
32 handled ones. Piglets positively handled at weaning produced shorter grunts than the other ones,  
33 regardless of the context of recording, which may indicate a more positive affect. During reunions  
34 with the static experimenter, a more positive HAR was associated with a decrease in vocal reactivity  
35 to human proximity. However, during reunions with the experimenter providing additional positive  
36 contacts and over the conditioning, proximity to the human systematically triggered shorter and  
37 higher pitched grunts, indicator of positive a emotional state. Results first show that changes in vocal  
38 structure are consistent with indicators of positive states in the presence of a human. Second, these  
39 changes are stronger when the human positively interact with the piglets, supposedly emphasizing a  
40 higher positive arousal state during these interactions. We show that vocalisation structure may be a  
41 promising indicator of the quality of human-pig relationship.

## 42 **Introduction**

43 The process of domestication was conducted to shape physiology and morphology of domestic  
44 animal species, but also their behaviour. It notably has shaped interspecific interactions between  
45 human and non-human animals, by improving animals' capacity to use human signals to adapt their  
46 behaviour both decreasing fearfulness toward humans and increasing attention toward humans  
47 (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is  
48 important for animal welfare. Therefore, studying human-animal interactions and their consequences  
49 to understand the mechanisms of emergence and maintenance of a positive human-animal  
50 relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three  
51 major aspects: the ability of an animal to control its mental and physiological stability (Broom,  
52 2011), the decrease of experiencing negatively perceived contexts and the increase in experiencing

53 positively perceived contexts and species-specific behaviors (Peterson et al., 1995; Weerd & Day,  
54 2009). A positive HAR is thought to be established through repeated positive interactions between  
55 the human and the non-human animal. Some of the mechanisms involved in this process are:  
56 accumulation of positive experiences through positive associative learning, modifications of  
57 cognitive biases, shaping expectations from the non-human animal toward the human. A positive  
58 HAR can be appreciated through behavioural and physiological measures, for example by assessing  
59 the expression of positive emotions [reviewed in (Rault et al. 2020)]. Several behavioural measures  
60 may help to define a positive HAR such as: short latency to approach and spatial proximity (Boivin  
61 et al., 2000; Schmied et al., 2008), body postures (Villain, Lanthony, et al., 2020) or play behaviour  
62 (Jerolmack, 2009). Contacts from a human such as stroking, may induce changes in body postures  
63 and exposition of body areas by the animal to the human, supposedly vulnerable [central neck area in  
64 cattle (Schmied et al. 2008), abdominal area in pigs (Rault et al., 2019)]. Such grooming solicitation  
65 may be markers of engagement, trust and motivation to interact with the human. In most cases, these  
66 behaviours are similar to those shown during intraspecific socio positive interactions, although there  
67 are some species specific behaviours [e.g., dog vs. wolf (Gácsi et al., 2005)]. Vocal behaviour may  
68 also help defining the quality of an HAR. First, some vocalisations type have been associated with  
69 positive interactions with humans, for example the cat – human communication : purring is thought  
70 to be derived from mother pup communication during nursing and is observed associated with care  
71 solicitation from humans; meowing, which is not observed during intra specific interactions is  
72 thought to emerge from associative learning during cat – human interactions (Brown & Bradshaw,  
73 2014). This shows that HAR may elicit specific vocalisations from the non human animal toward the  
74 human. Second, vocalisation structure is known to carry markers of the emotional states in several  
75 bird and mammal species (Briefer, 2012, 2020) and markers of emotional valence (positive versus  
76 negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Since  
77 positive or negative HAR is likely to affect the emotional state of animals, it is likely that it may be  
78 reflected in the structure of the produced vocalisations.

79 In pigs, diversified evidence attest the possibility of a positive HAR. Animals may be handled by  
80 humans providing regular additional positive contacts, leading to the expression of a positive  
81 perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests  
82 showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et  
83 al., 2015b). Pigs may recognise a human providing positive contacts compared to an unfamiliar one  
84 and adapt their behaviour accordingly (Brajon et al., 2015c). Pigs may be sensitive to human voice  
85 and respond accordingly (Bensoussan et al., 2019, 2020). Pigs vocalisations are diverse and linked to  
86 their emotional states, attested by the use of positive or negative call types (Briefer et al., 2019, 2022;  
87 Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related

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

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

## 108 **Methods**

### 109 Ethical note

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

## 116 Subjects and housing conditions

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

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

## 128 Treatment: positive handling at weaning

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

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

135 - **Positively handled piglets piglets (H+ piglets):** Experimental piglets from the 10 other rearing  
136 pens, housed in another room, received the same daily care given by the same stockperson as for H  
137 piglets. They additionally received repeated sessions of additional human contacts. Each pen of three  
138 piglets received 29 sessions of **10 min** , from day 28 (weaning) until day 39, occurring five days a  
139 week. Three sessions per day were performed (except on the day of weaning during which only two  
140 were done with a two-hour break in between). Each session took place in the rearing pen and the  
141 order of the interventions in the pens was balanced across days. The handling procedure, using gentle  
142 tactile contacts is described in supplementary material of Villain et al. (2020) and was similar to  
143 Tallet et al. (2014). **Briefly, the behaviour of the human toward the piglet was adapted to the reaction**  
144 **of each animal and included four steps: (1), the handler hold out the hand towards the animal; (2) if**  
145 **the piglet did not move away, the handler tried to touch it; (3) if the piglet accepted being touched,**  
146 **the handler softly stroked it along the body with the palm of her hand; and (4) once it accepted being**

147 stroked, the handler scratched it along the body with her fingers. Scratching consisted in rubbing the  
148 skin of the piglets with the finger tips and applying more pressure than stroking. No specific body  
149 part of the piglets was more considered than another. Two experimenters ('AV' and 'AH') performed  
150 these sessions (both women, both between 1.70-1.73 m tall, with a balanced number of pens  
151 attributed to each of them). The experimenters wore the same blue overalls and green boots each  
152 time they interacted with the piglets. The experimenters tried to imitate each others behaviours  
153 (remote video monitoring) to decrease variability.

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

### 158 Conditioning: sessions of additional positive contacts with 159 (un)familiar human

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

165 Briefly, all piglets were individually trained to learn to associate two different stimuli with the  
166 arrival of two different (pseudo)-social partners: either two pen mates (partner = Conspecifics) or a  
167 familiar human (partner = Human). When entering the room, the piglets and the partner(s) would  
168 remain in the room for 2 min. Specifically, when the human was the partner, the human entered, sat  
169 on a bucket and positively interacted with the piglet for 2 min, in the same manner as additional  
170 contacts was provided to the H+ piglets during the previous period (see above section) (figure 1).  
171 Therefore, at the beginning of the conditioning, H+ piglets were already familiar with the human and  
172 procedure, whereas H piglets were unfamiliar with the human. During the conditioning, the same  
173 sessions occurred in both treatment groups (H and H+). After the conditioning, all piglets were  
174 familiar with the human, but treatment groups had a different time of exposure to them. Sessions of  
175 reunions with social partners were not studied here because they were part of an analysis on vocal  
176 expression of positive anticipation reported earlier (Villain, Hazard, et al., 2020).

177 For every second trial, the 2 min reunions with the human were analysed by the same person: trials  
178 number 2, 4, 6, 8, 10 and 11 (see behavioural analyses section).

## 179 Standard Isolation/Reunion Tests with a static and silent human

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

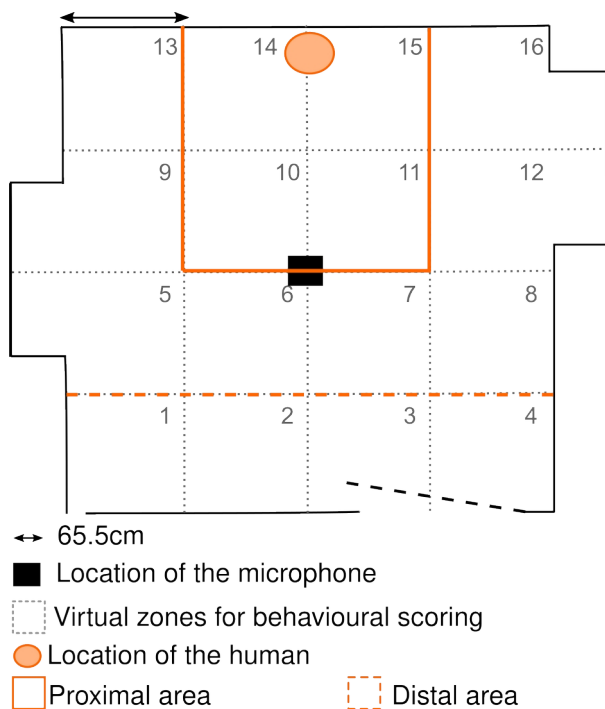


Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1, 2, 3, 4) were defined, using the location of the human as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

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

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

Table 1: Ethogram.

Behaviour	Description
No. zones crossed <sup>1,2</sup>	The number of times the piglet crossed a virtual zone
No. approaches H <sup>1</sup>	Number of times the piglets entered the proximal area
Time watching H <sup>1,2</sup>	The amount of time the piglet spent watching the human
Latency to contact H <sup>1,2</sup>	The latency to the first contact of the human by the piglet
No. looks toward H <sup>1,2</sup>	The number of times the piglet turned its head toward the human
No. looks at walls and doors <sup>1</sup>	The number of times the piglet looked at walls and doors
Time watching walls and doors <sup>1</sup>	The amount of times the piglet watched walls and doors
Time in proximal area <sup>1,2</sup>	The amount of time the piglet spent in the proximal area
Time in distal area <sup>1,2</sup>	The amount of time the piglet spent in the distal area
Time in contact H <sup>1,2</sup>	The amount of time the piglet investigated the human
Time investigating floor <sup>1,2</sup>	The amount of time the piglet investigated the floor
No. contacts H <sup>2</sup>	Number of times the piglet was in contact with the human (initiated by the piglet or the human)

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

196

## 197 Acoustic monitoring and analyses

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

210 A spectro-temporal analysis was performed with custom-written codes using the Seewave R  
 211 package (Sueur et al., 2008) implemented in R (R Core Team, 2022). We first studied the spectral  
 212 properties of the remaining background noise of the experimental room (electric noises and  
 213 remaining low frequency noises from the rest of the building), using 20 examples of 0.5 second  
 214 fragments and compared it with the general frequency range of the grunts. To avoid measuring  
 215 masking effect of the background noise, grunts were filtered using a 0.2-8 kHz bandpass filtering  
 216 (‘fir’ function). As a consequence, all results presented in this study are on a 0.2-8 kHz frequency  
 217 range, and no conclusions on frequency components of grunts below this 200 Hz threshold are drawn



218 [here](#). Several acoustic parameters were then extracted from each grunt. To measure grunt duration, a  
 219 5% to maximal amplitude threshold was used ('timer' function). After normalisation, the following  
 220 spectral parameters were calculated using the 'specprop' function (FFT with Hamming window,  
 221 window length = 512, overlap = 50%): mean (Q50), first (Q25) and third (Q75) quartiles,  
 222 interquartile range (IQR), centroid and standard deviation (all in Hz). The grunt dominant frequency  
 223 (in kHz) was also calculated ('dfreq', 50% overlapping FFTs, window length = 512), which is the  
 224 mean over the grunt duration of the frequencies of highest energy of each window. Frequency peaks  
 225 were detected and the minimal and maximal peaks were kept as descriptors ('fpeaks' function,  
 226 window length = 512, peak detection threshold = 10% of the normalized amplitude). Measures of  
 227 noisiness and entropy of the grunts were assessed using: Shannon entropy (sh), Spectral Flatness  
 228 (Wiener entropy, sfm) and Entropy (H) [combining both Shannon and Temporal envelop entropy,  
 229 length = 512, Hilbert envelop). Two vocal scores were used: the logarithm of grunt duration and a  
 230 built-in spectral vocal score with all spectral parameters (see below). A table describing mean and  
 231 range of variation of each acoustic parameter in the relevant contexts of the study is available in the  
 232 supplementary material (table S4).

## 233 Statistical analyses

### 234 ***Behavioural and vocal response scores***

235 All measures extracted from videos or sound analysis are named parameters throughout the text.  
 236 The symmetrical distribution of parameters (behavioural on the one hand and acoustic on the other  
 237 hand) was visually inspected, and linear transformations were computed when necessary to reach  
 238 symmetrical distribution (see tables 2, 3, 4). When this criteria was reached, Principal Component  
 239 Analyses (PCA, one for the behavioural analysis and one for the spectral acoustic analysis) were  
 240 performed using several parameters to build scores ['dudi.pca' function from 'ade4' R package  
 241 (Dray & Dufour, 2007) and 'inertia.dudi' function to extract the loadings]. These scores were then  
 242 used as statistical variables. Indeed, PCAs are generally used to reduce the number of variables  
 243 included in statistical models. It also generates quantifiable global descriptors of behaviours or  
 244 acoustic structure, since correlated parameters usually load on the same PC (McGregor, 1992). All  
 245 PCs having an eigenvalue above one were kept and constituted response scores of behavioural  
 246 ('ReuPCs' and 'CondPCs' in table 2 and 3 respectively) and vocal ('VocPCs', table 4) parameters.  
 247 Only the duration of grunts was kept separated from the spectral parameters to keep it as a temporal  
 248 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
No. of zones crossed (sqrt)	24.177	<b>-55.843</b>	-0.435
No. approaches H (ln)	<b>47.748</b>	-30.163	0.578
Time watching H (ln)	<b>-52.914</b>	-7.422	25.585
Latency to contact H (ln)	<b>-64.232</b>	-0.464	1.688
No. looks toward H (sqrt)	-7.787	<b>-43.721</b>	31.633
Time watching room	-32.048	-13.581	-6.238
No. looks at walls and doors (sqrt)	3.524	<b>-72.408</b>	-2.027
Time in proximal area (sqrt)	<b>69.96</b>	-0.156	9.584
Time in distal area (sqrt)	<b>-46.416</b>	-12.437	-1.215
Time in contact H (sqrt)	<b>61.041</b>	3.586	24.183
Time spent investigating floor	11.868	-7.503	<b>-42.265</b>

249

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)	<b>80.23</b>	2.542	-0.112
Time in distal area (ln)	-33.826	8.547	30.789
Number of contacts H (ln)	<b>78.55</b>	6.476	2.288
Time in contact H (ln)	<b>86.625</b>	0.715	-0.369
No. looks toward H (ln)	-2	<b>79.898</b>	-0.745
Time watching H (ln)	-6.757	<b>65.67</b>	-10.325
No. of zones crossed (sqrt)	0.129	33.599	<b>48.457</b>
Time spent investigating floor	0.006	<b>-49.286</b>	14.205
Latency to contact H	<b>-81.01</b>	-0.248	-2.83

250

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 <sup>1</sup>	-13.558	<b>53.557</b>	2.220
Min frequency peak <sup>1</sup> (ln)	-0.349	<b>58.758</b>	24.236
Max frequency peak <sup>1</sup>	<b>-43.023</b>	8.760	-9.537
Mode <sup>2</sup> (ln)	-0.522	<b>66.248</b>	19.268
Mean <sup>2</sup> (ln)	<b>-95.092</b>	-2.295	2.028
Q50 <sup>2</sup> (ln)	<b>-85.278</b>	0.280	-0.093
Q25 <sup>2</sup> (ln)	<b>-52.360</b>	19.327	0.985
Q75 <sup>2</sup> (sqrt)	<b>-88.925</b>	-4.645	2.309
Centroid <sup>2</sup> (ln)	<b>-95.092</b>	-2.295	2.028
Sd <sup>2</sup>	<b>-64.484</b>	-11.303	7.680
IQR <sup>2</sup>	<b>-87.981</b>	-5.851	2.640
Sfm <sup>3</sup> (sqrt)	<b>-94.344</b>	-3.189	0.962
Sh <sup>3</sup> (sqrt)	<b>-96.087</b>	-0.785	-0.175
H <sup>3</sup>	<b>-88.205</b>	-1.059	-1.063
Skewness <sup>4</sup>	28.032	-18.010	<b>48.652</b>
Kurtosis <sup>4</sup>	22.973	-16.241	<b>50.615</b>

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

## 251 *Statistical models*

252 All statistics were carried out on R (R Core Team, 2022). Linear mixed effect models [*lmer*  
253 function, *lme4* R package (Bates et al., 2014)] were built when tested variables were linear  
254 (behavioural and vocal scores, grunt duration) and one binomial generalized mixed effect model was  
255 built for binary parameters (occurrence of missed contacts initiated by human during the  
256 conditioning). The following subsections describe how models were built for each type of tests. In all  
257 models described below, the identity of the replicate ('1' or '2') was used as an interacting fixed

258 factor, since the experiment was run in two identical replicates on two independent groups. The  
 259 identity of the human ('AH' or 'AV') was used as interacting fixed factor in all models described  
 260 below, since two experimenters were involved in the positive handling at weaning and in the session  
 261 of additional positive contacts of the conditioning (but always the same human was attributed to a  
 262 given piglet). The piglet was used as random factor to take into account the within-subject design.  
 263 All explanatory variables used in the models and interactions between them were built in respect to  
 264 the experimental design and to allow biological interpretations. As a consequence, not all  
 265 interactions between all variables were made. They are fully explained in the subsequent sections.

## 266 Isolation/Reunion tests

267 The aim of this part was to test the effect of the positive handling at weaning treatment (H vs. H+  
 268 piglets) and additional human contacts during sessions of the conditioning on the piglet's reaction to  
 269 human presence. Since the same Isolation/Reunion test was repeated before and after the  
 270 conditioning, we used the variable 'Conditioning time' as a two level interacting factor ('before' or  
 271 'after' conditioning, referred as "Time" in the models) to test the effect of the conditioning. Piglets  
 272 spacial behaviour and proximity to the human was studied only during the reunion phase with the  
 273 human that followed the isolation phase. Model\_1 was computed:

```
274 Model_1 <- lmer (ReuPCs ~ Treatment*Time + Treatment*Replicate +
275 Treatment*HumanID + Time*Replicate + Time*HumanID + (1 | pigletID), data=
276 data_Behaviour_Reunion).
```

277 Concerning the analysis of vocal behaviour, the isolation phase represents a negative social context  
 278 for the piglets and may be used as a negative control when monitoring the effect of human presence  
 279 on vocal expression of emotional states (Villain, Lanthony, et al., 2020). So, the two phases of the  
 280 test were used to study the three way interaction between treatment (H vs.. H+), phase of the test  
 281 (isolation vs.. reunion) and time of the conditioning (before vs.. after). The following model\_2 was  
 282 computed:

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

286 To go further, only the reunion phase was kept and a proximity variable was added. Indeed, the  
 287 piglet could vocalise either when close to human or away from them and this spatial proximity was  
 288 demonstrated as an important factor of changes of vocal features (Villain et al. 2020b). Thus, a two  
 289 level proximity factor was built: either '1' when the piglet was in the proximal area (figure 1) or '0'  
 290 when it was elsewhere in the room. The following model\_3 was computed:

```

291 Model_3 <- lmer (Vocal response score ~ Treatment*Time*InProxArea +
292 Treatment*HumanID + InProxArea*HumanID + Treatment*Replicate +
293 InProxArea*Replicate + Time*Replicate + Time*HumanID + (1 | pigletID/Time), data
294 = data_Vocal_Reunion).

```

## 295 **Conditioning trials**

296 The aim was to study the evolution of human-piglet relationship over the conditioning [the variable  
297 ‘Trial number’, used as a continuous variable, referred as “Trial” in the models]. The effect of  
298 treatment (positively handled at weaning H+ piglets or non handled H piglets) was tested as an  
299 interacting factor with Trial. Trial was also used as a random slope to take into account individual  
300 trajectories (Schielzeth and Forstmeier 2009). The following model\_4 was built to test the  
301 behavioural response scores CondPCs (lmer) and the occurrence of missed contact initiated by the  
302 human during a session (presence/absence, binomial model, glmer):

```

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

```

306 For the analysis of vocal response scores, similarly to the Isolation/Reunion test, the piglet could  
307 vocalise either when close to the human or away from them. We thus added the proximity factor in  
308 the analysis of vocal response variables. The following model\_5 was built :

```

309 Model_5 <- lmer (VocPCs ~ Trial*Treatment*InProxArea+ Trial*HumanID +
310 Trial*Replicate + Treatment*Replicate + Treatment*HumanID + HumanID*InProxArea +
311 Replicate*InProxArea + (1+ Trial | pigletID), data= data_Vocal_Conditioning).

```

## 312 **Model validation and statistical tests**

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

324 **Results**325 Effect of positive handling at weaning and conditioning on piglets'  
326 reaction to human presence (Isolation/Reunion tests)327 ***Piglets that were not handled at weaning express a similar behavioural proximity***  
328 ***to a human after a positive conditioning as the positively handled ones.***

329

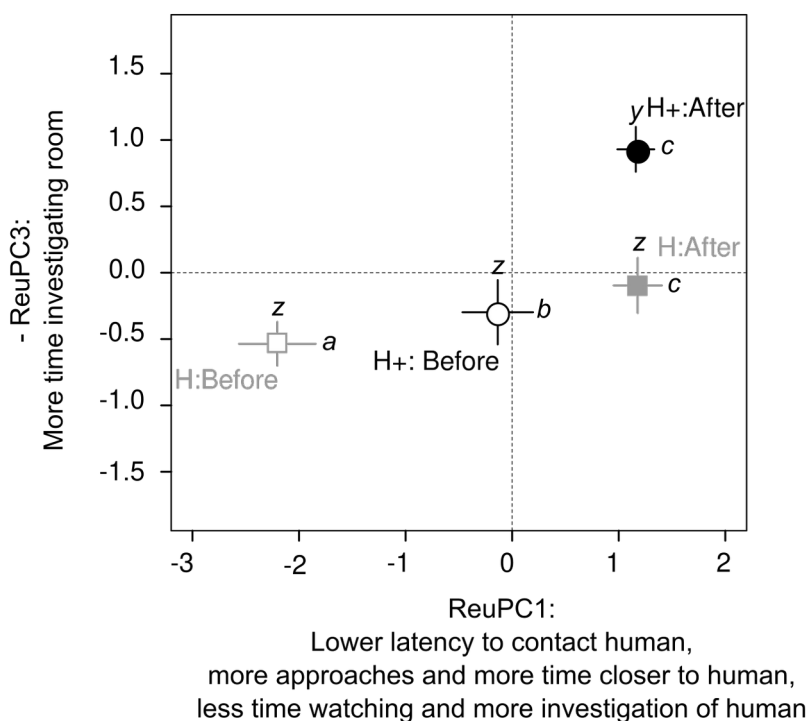


Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean  $\pm$  SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey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

330 The interaction between the treatment (positively handled piglets at weaning (H+) or not (H) and  
331 the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3  
332 ( $\chi^2_1 = 28.0$ ,  $p < 0.001$ , and  $\chi^2_1 = 3.7$ ,  $p = 0.05$  respectively, figure 2) but not for ReuPC2 ( $\chi^2_1 <$   
333  $0.001$ ,  $p = 0.99$ , supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was higher  
334 after the conditioning than before (H: after – before, t.ratio = 12.1,  $p < 0.001$ , H+: after – before  
335 t.ratio = 11.0,  $p < 0.001$ ) and that before the conditioning, piglets that were positively handled at  
336 weaning had significantly higher ReuPC1 than non handled piglets (Before, H – H+: t.ratio = -2.1,  $p$   
337  $< 0.001$ ), but not after (After, H – H+: t.ratio = 0.02,  $p = 1.0$ ). According to the loadings, this means

338 that piglets that were positively handled at weaning had a lower latency to contact the human,  
 339 approached them more often and spent more time close to and investigating the human (ReuPC1)  
 340 than non handled piglets, before the conditioning. This score increased after the conditioning and no  
 341 evidence of a difference between treatments after the conditioning was found (figure 2). Post hoc  
 342 tests on ReuPC3 showed a significant effect of the conditioning time only in piglets that were  
 343 positively handled at weaning (H+: after – before, t.ratio = 5.2,  $p < 0.001$ , H: after – before, t.ratio =  
 344 2.6,  $p = 0.06$ ). No difference in ReuPC3 was found between treatments before the conditioning  
 345 (Before: H – H+, t.ratio = -0.75,  $p = 0.87$ ), whereas positively handled piglets had a higher -ReuPC3  
 346 after the conditioning than before (After : H – H+, t.ratio = -3.2,  $p = 0.009$ ). According to the  
 347 loadings, this means that after the conditioning, piglets that were positively handled at weaning  
 348 expressed more investigation of the room after the conditioning than non handled piglets. No  
 349 evidence of any effect on ReuPC2 was found (table S2).

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

352 Using the isolation phase as a negative control we could compare the effect of the phase of the test  
 353 (Isolation vs. Reunion with the human), taking into account the conditioning time (before or after the  
 354 conditioning) and the treatment. No evidence of any effect of neither the three way interaction ( $\chi^2_1 <$   
 355 0.62,  $p > 0.43$ ) nor two way interactions of interest was found (treatment: phase, conditioning  
 356 time:phase, conditioning time: treatment interactions :  $\chi^2_1 < 3.5$ ,  $p > 0.06$ , table S2) in any of the  
 357 scores.

358 Regardless of the treatment, single effects of the phase of the test were significant for grunt  
 359 duration and all AcPCs ( $\chi^2_1 > 6.6$ ,  $p < 0.01$ , table S1). During the reunion phase with the human,  
 360 grunts were shorter (estimates of  $\log(\text{duration})$ [95% CI] : -1.32[-1.37;-1.26] vs. -1.06[-1.12;-1.00]),  
 361 had a higher frequency range, higher bandwidth and a higher noise component (-VocPC1:  
 362 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[-  
 363 0.65;-0.28]) and their spectrum had a higher skewness and kurtosis (VocPC3: -0.25[-0.37;-0.14] vs. -  
 364 0.11[-0.23;0.01] ), compared to the isolation phase.

365 Regardless of the phase of the test, single effects of treatment were found for grunt duration and -  
 366 VocPC3 ( $\chi^2_1 = 5.5$ ,  $p = 0.02$  and  $\chi^2_1 = 4.9$ ,  $p = 0.03$  respectively, table S2). Grunts produced by  
 367 positively handled at weaning piglets were shorter (estimates of  $\log(\text{duration})$ [95% CI]: -1.25[-1.32;-  
 368 1.19] vs. -1.12[-1.2;-1.1], table S3), and differed in -VocPC3 scores, describing the shape of the

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

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

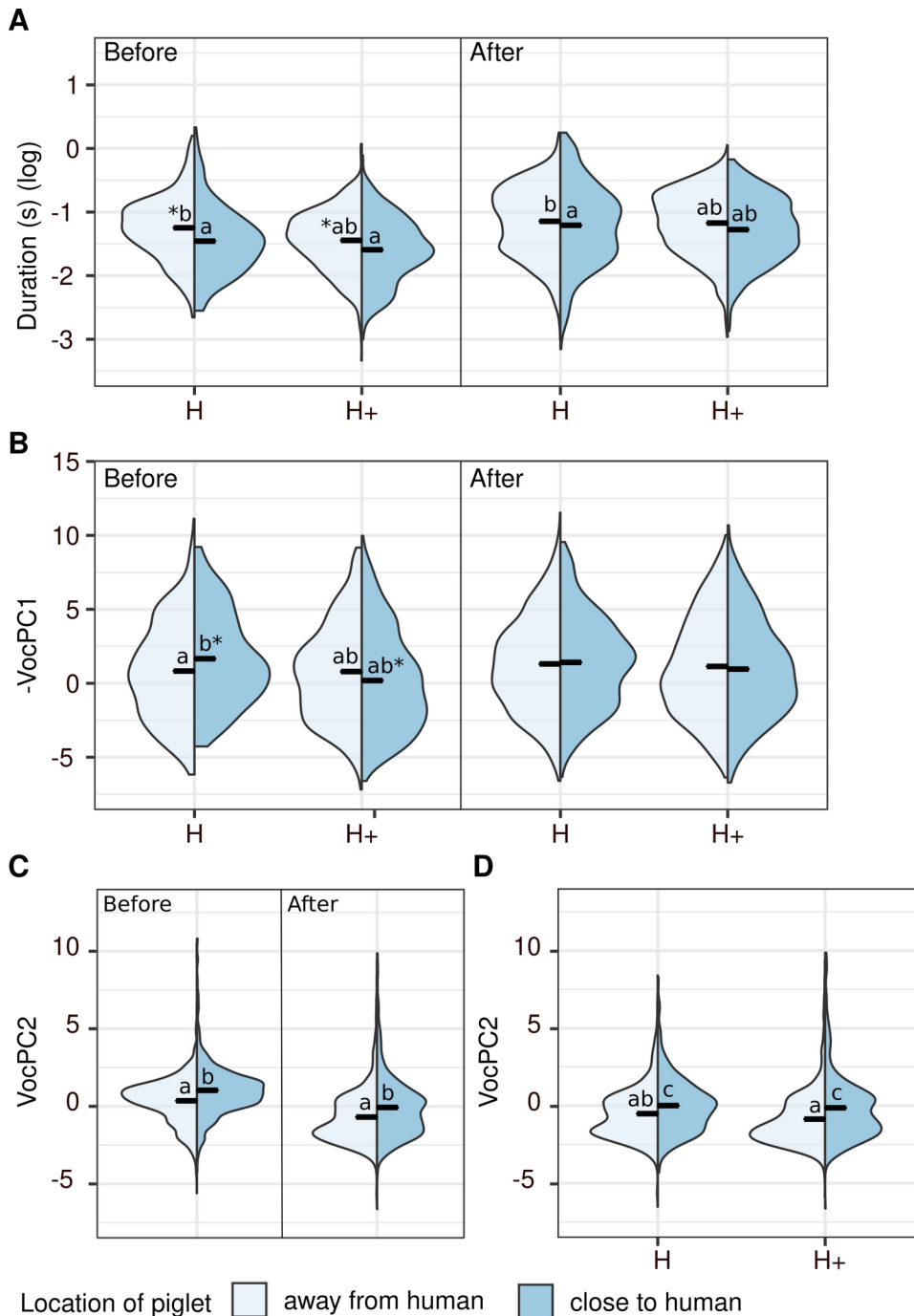


Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect of conditioning (before or after), treatment (H or H+) and location of the piglet relatively to the human (close: dark blue or away from them: light blue). Violin plots representing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



372 During the 5 min reunion, the piglet was scored either as close to the human or away from them.  
373 The three way interaction of the conditioning time, the treatment and the location was significant for  
374 grunt duration, -VocPC1 and VocPC3 ( $\chi^2_1 > 4.9$ ,  $p < 0.03$ ). Post hoc tests revealed that grunts  
375 produced closer to the human were shorter than the ones produced further away, but only in piglets  
376 that were not positively handled at weaning, effect being stronger before the conditioning than after  
377 it (H piglets: away – close,  $z.ratio = 6.3$ ,  $p < 0.001$  before and  $z.ratio = 4.1$   $p < 0.001$  after the  
378 conditioning; H+ piglets: away – close  $z.ratio < 1.98$   $p > 0.19$ , figure 3A). -VocPC1 was higher, i.e.  
379 grunts had a higher frequency range, bandwidth and were noisier when produced closer to the human  
380 than further away, but only in non handled piglets and before the conditioning (H piglets: away –  
381 close,  $z.ratio = -3.34$ ,  $p = 0.005$  before and  $z.ratio = -1.23$   $p = 0.61$  after the conditioning; H+ piglets:  
382 away – close,  $z.ratio < 0.36$   $p > 0.21$ , figure 3B). For VocPC2, the three way interaction did not  
383 reach significance ( $\chi^2_1 = 3.3$ ,  $p = 0.07$ ), so only subsequent two way interactions were considered  
384 (post hoc tests on the three way interaction can be found in supplementary, tables S1 to S3). For  
385 VocPC2, significant two way interactions were found between the conditioning time and the location  
386 ( $\chi^2_1 = 10.3$ ,  $p = 0.001$ ) on the one hand, and between the location and the treatment ( $\chi^2_1 = 4.2$ ,  $p =$   
387  $0.04$ ) on the other hand. Post hoc tests revealed that grunts produced closer to the human had a  
388 higher VocPC2, meaning they had a higher pitch, effect being stronger before the conditioning than  
389 after (before: away – close,  $z.ratio = -6.12$ ,  $p < 0.001$ ; after: away – close,  $z.ratio = -2.88$ ,  $p = 0.004$ ,  
390 figure 3C). The increase in VocPC2 with the location was greater for non handled piglets than  
391 positively handled piglets (H piglets: away – close,  $z.ratio = -5.54$ ,  $p < 0.001$ ; H+ piglets: away –  
392 close,  $z.ratio = -3.82$ ,  $p = 0.001$ , figure 3D). The last two-way interaction of interest between the  
393 conditioning time and the treatment did not reach significant level ( $\chi^2_1 = 0.80$ ,  $p = 0.37$ ). For  
394 VocPC3, post hoc tests did not reach significant levels ( $|z.ratio| < 2.3$   $p > 0.09$  for any comparison) .

395

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

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

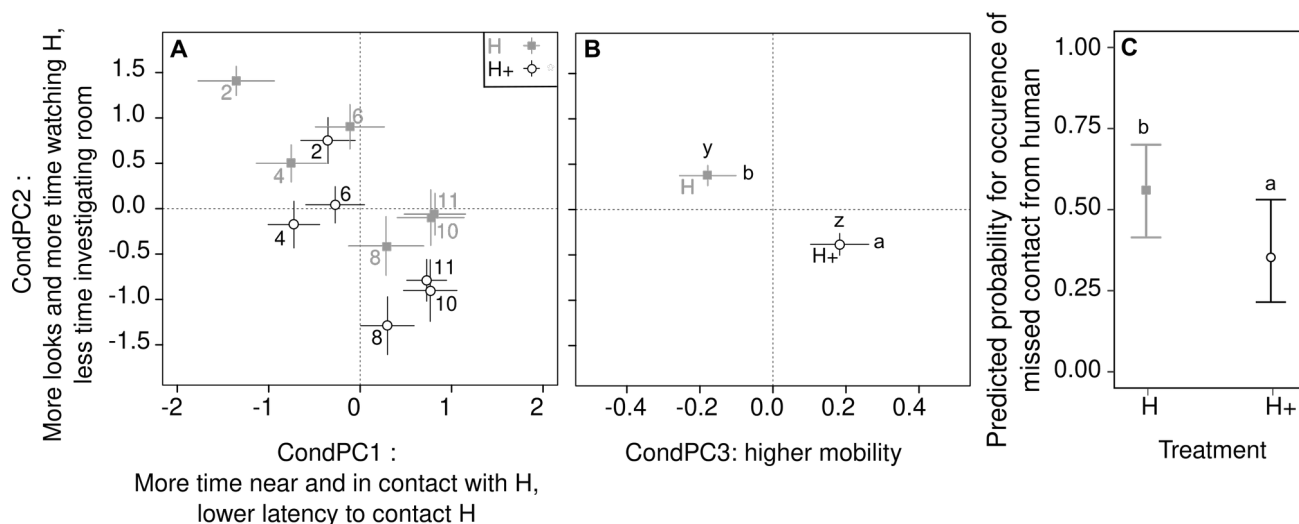


Figure 4: Behavioural variation of responses of piglets according to the sessions of additional positive contacts of the conditioning (A), and to the treatments (B, C). (A,B) Mean  $\pm$  SE per group. Numbers in (A) refers to the trial number of the conditioning. Higher CondPC1 and lower CondPC2 over time (single effect of trial number, A). Higher CondPC2 in H piglets than H+ piglets regardless of time (single effect of treatment, A). Higher CondPC3 and lower CondPC2 in H+ piglets than H piglets (single effect of treatment, B). (C) Mean estimates  $\pm$  95% confidence interval from generalized mixed effect model. Lower probability of occurrence of missed contact by the human in H+ than H piglets (single effect of treatment). Full statistical report is available as supplementary material (tables S1 et S2 for statistical tests, table S3 for model estimates).

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

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

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

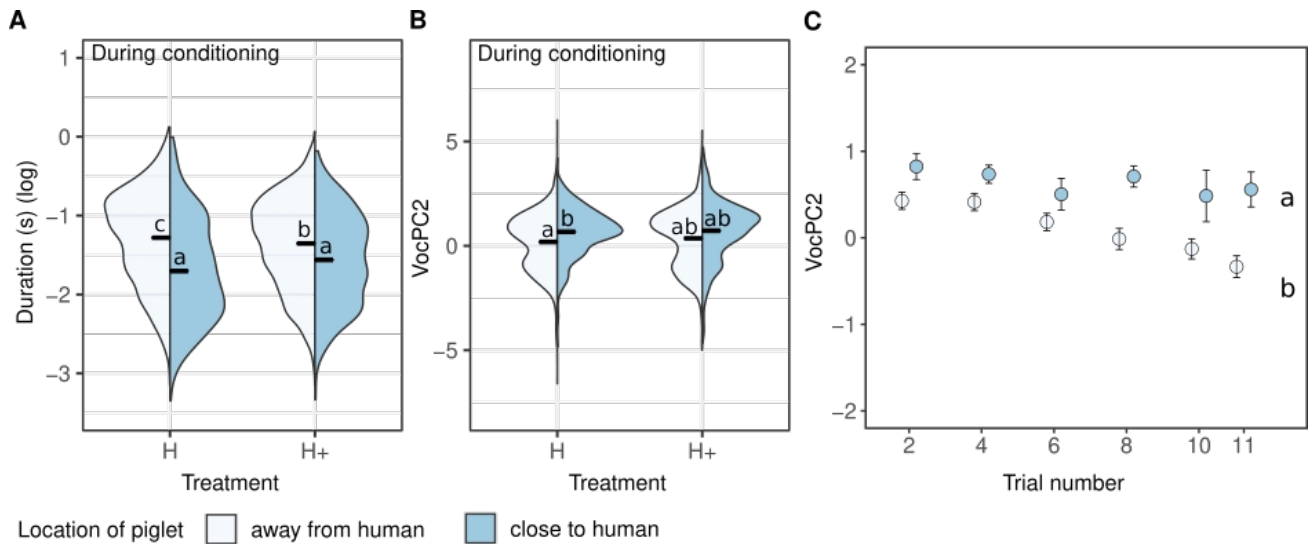


Figure 5: Vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human 'close': dark blue) or elsewhere in the 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).

416

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

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

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

438

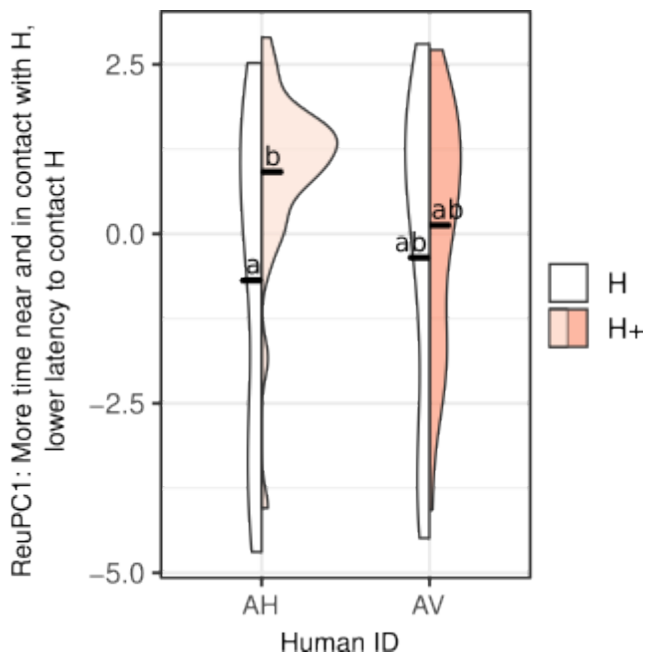


Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Values with no common letters differ significantly. Full statistical report is available as supplementary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

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

443 During the reunions of the Isolation/Reunion test, the interaction between treatment and human  
 444 identity was significant for the first behavioural proximity score (ReuPC1,  $\chi^2_1 = 6.01$ , p = 0.01) but  
 445 not the others (ReuPC2 and ReuPC3 ( $\chi^2_1 < 1.98$ , p > 0.16, table S1). The effect of treatment on  
 446 ReuPC1 was higher when piglets were handled by the human ‘AH’ (H vs. H+, AH: t.ratio = -4.77, p  
 447 < 0.001, figure 6). When the human ‘AV’ handled the piglets, for which ReuPC1 scores exhibited  
 448 intermediate values, treatment was not significant (AV, H vs. H+: t.ratio = -1.33, p = 0.56). These

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

451 Interactions between the human identity and conditioning time were not significant, neither  
452 considering the reunions of the Isolation/Reunion test (ReuPCs,  $\chi^2_1 < 0.642$ ,  $p > 0.42$ , tables S1),  
453 neither the trial number during the session of additional positive contacts of the conditioning  
454 (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
<b>Reunion of the Isolation/Reunion test</b>							
VocPC2	humanID	AH	0.154	-0.119	0.427	$\chi^2_1 =$ 4.94	P = 0.03
		AV	-0.292	-0.571	-0.012		
<b>Sessions of additional positive contacts of the conditioning</b>							
-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*I nProxArea	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		

455

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

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

## 472 **Discussion**

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

### 489 Behavioural evidence of a rapid establishment of interest and 490 proximity toward a human providing additional positive contacts

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

493 (H and H+), as positively handled piglets expressed a higher attraction toward the human than non  
494 handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al.,  
495 2020). Second, this test showed that the conditioning increased the behavioural proximity toward the  
496 human of both positively handled and non handled piglets so that non handled piglets expressed a  
497 similar attraction toward the human as positively handled piglets. These results are in line with the  
498 behavioural results of the sessions of additional positive contacts. The analysis of piglets' behaviour  
499 every second sessions of the conditioning showed that, although positively handled and non handled  
500 piglets started with different degree of proximity toward the human (trials 2 and 4, CondPC1), then,  
501 over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human  
502 (CondPC1) and avoided less the human when the latter attempted to interact with them. So it seems  
503 that the conditioning process allowed non handled piglets to compensate the lack of positive  
504 handling before the conditioning and develop a similar proximity toward the human. Two minute  
505 daily sessions of additional positive contacts changed positively the perception of the human for the  
506 piglets, and thus their willingness to interact with them. Since no evidence of any interaction  
507 between time and treatment was found, no conclusion on differential developmental trajectories  
508 between treatments can be drawn, but a parallel development of the human-piglet relationship in  
509 both groups, when considering the proximity.

510 Beside behavioural proximity, piglets that were positively handled at weaning expressed more  
511 exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also  
512 observed during the sessions of additional positive contacts of the conditioning: positive handled  
513 piglets started with a higher score associated with investigation than non handled piglets (CondPC2)  
514 and it held over the conditioning. Piglets that were positively handled at weaning also expressed a  
515 higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an  
516 expression of natural foraging and disinterest from human contact, which may be a sign of positive  
517 welfare (Weerd & Day, 2009). In addition, this could also be interpreted in terms of attachment to  
518 the human. Indeed, attachment to a human may facilitate exploration of novel environments or  
519 objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may  
520 provide an environment secure enough for the piglets to explore their environment in the presence of  
521 the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al.,  
522 2009).

523 Overall, the behavioural monitoring showed that 2 min sessions of positive additional contacts per  
524 day are sufficient to increase proximity to a human to similar levels as when piglets were previously

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

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

### 533 Links between vocal expression and positive HAR

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

535 The social isolation phase of the Isolation /Reunion test, before any human entered the room, was  
536 associated with longer, lower pitched grunts with a downshifted frequency spectrum, whereas the  
537 reunion with a static human changed grunts structure to shorter, higher pitched with an upshifted  
538 frequency spectrum and this was observed in both handled and non handled piglets (H or H+) as well  
539 as before and after the conditioning. In terms of emotional indicators, similar changes in acoustic  
540 features of grunts were found in studies focusing on vocal markers of valence in pigs (Briefer et al.,  
541 2019, 2022; Friel et al., 2019; Villain, Hazard, et al., 2020), meaning that the reunion with a human,  
542 after a period of social isolation would be perceived as positive. However, this modulation of grunt  
543 structure was observed regardless of piglet experience with the human. It is possible that the reunion  
544 with an either neutral or familiar human, releasing piglets from total isolation could be perceived as  
545 positive by the piglets, as suggested in previous studies (Villain, Lanthony, et al., 2020).

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



555 category of affect, moods, and not only emotional expression. Indeed, as suggested by Schnall  
556 (2010), although emotions are short-term affects triggered by an external stimulus, moods, on the  
557 other hand, may be experienced on a longer term and may not be attributable to a specific stimulus.  
558 Although emotions and moods do not rely on the same time scale, they may interact with one  
559 another, and more studies are needed to understand their effects on vocal expression.

### 560 *A positive HAR affects vocal reactivity toward a static human*

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

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

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

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

590 ***Providing rewarding additional positive contacts triggers short and high pitched***  
591 ***grunts***

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

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

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

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

### 636 Effect of human identity on piglets' perception: perspectives on 637 HAR

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

650 human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering  
651 positive emotions and thus modifications of grunt structure. Several evidence exists in the literature  
652 that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al.,  
653 2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We  
654 may question the efficiency of different human features to generate a positive HAR. In our study,  
655 both humans that interacted with the piglets wear exactly the same clothes and standardized their  
656 tactile interactions toward the piglets before starting the study, and agreed on the rhythm and types of  
657 sounds (words, intonation) to use, to minimise generating variability although no systematic controls  
658 of the human behaviour or spectral feature of voices were performed here. It thus remains unclear  
659 whether experimenters interacted differently or if they were initially perceived differently by piglets.  
660 Our results show that the identity of the human may modulate piglet proximity and vocal behaviour  
661 but the design of this experiment does not allow to find the causes of these observations (behaviour,  
662 voice characteristics, or even odour profile). Thus, more studies of human features that are most  
663 likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In  
664 addition, studying human-piglet relationship in a more systematic way, as in other domestic species,  
665 for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech  
666 (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies  
667 of interspecific relationships. However, the influence of human identity did not modify the general  
668 outcomes of our study, but only decreased some effects, suggesting that this variability does not  
669 modify the main results, but should be considered in future studies.

670

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

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

## 684 **Authors contributions**

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

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## 695 **Data availability**

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

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702 **non colored clean version**

# 703 **The use of pigs vocalisation structure to assess the quality of** 704 **human-pig relationship**

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## 714 **Key words**

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

## 717 **Abstract**

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

722 animal biology and emotional states (social, spatial and postural behaviours, physiological and  
723 cognitive states). Growing evidence shows that acoustic features of animal vocalisations may be  
724 indicators of emotional states. Here, we tested the hypothesis that vocal structure may indicate the  
725 quality of HAR. At weaning, 30 piglets were positively handled by an experimenter who talked to  
726 and physically interacted with them three times a day, while 30 other piglets only received the  
727 contact necessary for proper husbandry. After two weeks, we recorded the behaviours and  
728 vocalisations produced in the presence of the static experimenter for 5 min. We repeated this test  
729 two weeks later, after a conditioning period during which human presence with additional positive  
730 contacts was used as a reward for all piglets. We hypothesized this conditioning period would lead to  
731 a positive human-piglet relationship for all piglets. As expected, piglets that were positively handled  
732 at weaning expressed a higher attraction toward the experimenter, and, after the conditioning, piglets  
733 that were not positively handled at weaning expressed a similar level of attraction than the positively  
734 handled ones. Piglets positively handled at weaning produced shorter grunts than the other ones,  
735 regardless of the context of recording, which may indicate a more positive affect. During reunions  
736 with the static experimenter, a more positive HAR was associated with a decrease in vocal reactivity  
737 to human proximity. However, during reunions with the experimenter providing additional positive  
738 contacts and over the conditioning, proximity to the human systematically triggered shorter and  
739 higher pitched grunts, indicator of positive a emotional state. Results first show that changes in vocal  
740 structure are consistent with indicators of positive states in the presence of a human. Second, these  
741 changes are stronger when the human positively interact with the piglets, supposedly emphasizing a  
742 higher positive arousal state during these interactions. We show that vocalisation structure may be a  
743 promising indicator of the quality of human-pig relationship.

## 744 **Introduction**

745 The process of domestication was conducted to shape physiology and morphology of domestic  
746 animal species, but also their behaviour. It notably has shaped interspecific interactions between  
747 human and non-human animals, by improving animals' capacity to use human signals to adapt their  
748 behaviour both decreasing fearfulness toward humans and increasing attention toward humans  
749 (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is  
750 important for animal welfare. Therefore, studying human-animal interactions and their consequences  
751 to understand the mechanisms of emergence and maintenance of a positive human-animal  
752 relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three  
753 major aspects: the ability of an animal to control its mental and physiological stability (Broom,  
754 2011), the decrease of experiencing negatively perceived contexts and the increase in experiencing

755 positively perceived contexts and species-specific behaviors (Peterson et al., 1995; Weerd & Day,  
756 2009). A positive HAR is thought to be established through repeated positive interactions between  
757 the human and the non-human animal. Some of the mechanisms involved in this process are:  
758 accumulation of positive experiences through positive associative learning, modifications of  
759 cognitive biases, shaping expectations from the non-human animal toward the human. A positive  
760 HAR can be appreciated through behavioural and physiological measures, for example by assessing  
761 the expression of positive emotions [reviewed in (Rault et al. 2020)]. Several behavioural measures  
762 may help to define a positive HAR such as: short latency to approach and spatial proximity (Boivin  
763 et al., 2000; Schmied et al., 2008), body postures (Villain, Lanthony, et al., 2020) or play behaviour  
764 (Jerolmack, 2009). Contacts from a human such as stroking, may induce changes in body postures  
765 and exposition of body areas by the animal to the human, supposedly vulnerable [central neck area in  
766 cattle (Schmied et al. 2008), abdominal area in pigs (Rault et al., 2019)]. Such grooming solicitation  
767 may be markers of engagement, trust and motivation to interact with the human. In most cases, these  
768 behaviours are similar to those shown during intraspecific socio positive interactions, although there  
769 are some species specific behaviours [e.g., dog vs. wolf (Gácsi et al., 2005)]. Vocal behaviour may  
770 also help defining the quality of an HAR. First, some vocalisations type have been associated with  
771 positive interactions with humans, for example the cat – human communication : purring is thought  
772 to be derived from mother pup communication during nursing and is observed associated with care  
773 solicitation from humans; meowing, which is not observed during intra specific interactions is  
774 thought to emerge from associative learning during cat – human interactions (Brown & Bradshaw,  
775 2014). This shows that HAR may elicit specific vocalisations from the non human animal toward the  
776 human. Second, vocalisation structure is known to carry markers of the emotional states in several  
777 bird and mammal species (Briefer, 2012, 2020) and markers of emotional valence (positive versus  
778 negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Since  
779 positive or negative HAR is likely to affect the emotional state of animals, it is likely that it may be  
780 reflected in the structure of the produced vocalisations.

781 In pigs, diversified evidence attest the possibility of a positive HAR. Animals may be handled by  
782 humans providing regular additional positive contacts, leading to the expression of a positive  
783 perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests  
784 showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et  
785 al., 2015b). Pigs may recognise a human providing positive contacts compared to an unfamiliar one  
786 and adapt their behaviour accordingly (Brajon et al., 2015c). Pigs may be sensitive to human voice  
787 and respond accordingly (Bensoussan et al., 2019, 2020). Pigs vocalisations are diverse and linked to  
788 their emotional states, attested by the use of positive or negative call types (Briefer et al., 2019, 2022;  
789 Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related

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

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

## 810 **Methods**

### 811 Ethical note

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



## 818 Subjects and housing conditions

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

823 One piglet had to be excluded from our sample size to receive care/medication due to health issues  
824 independent from the experiment. From weaning at 28 days of age, piglets from the same litter and  
825 having similar weight (<1 kg difference) were housed by three in a 1.2 x 1.3m pen on plastic  
826 duckboard. Wooden panels were used to visually isolate pens. One metal chain per pen was used for  
827 enrichment. Food and water were available *ad libitum*. Artificial lights were turned on from 8:00 to  
828 17:00 and temperature was maintained between 26 and 27 °C. The experiment was carried out in two  
829 replicates and two identical rearing rooms were used (5 pens per room per replicate).

## 830 Treatment: positive handling at weaning

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

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

837 - **Positively handled piglets piglets (H+ piglets):** Experimental piglets from the 10 other rearing  
838 pens, housed in another room, received the same daily care given by the same stockperson as for H  
839 piglets. They additionally received repeated sessions of additional human contacts. Each pen of three  
840 piglets received 29 sessions of 10 min , from day 28 (weaning) until day 39, occurring five days a  
841 week. Three sessions per day were performed (except on the day of weaning during which only two  
842 were done with a two-hour break in between). Each session took place in the rearing pen and the  
843 order of the interventions in the pens was balanced across days. The handling procedure, using gentle  
844 tactile contacts is described in supplementary material of Villain et al. (2020) and was similar to  
845 Tallet et al. (2014). Briefly, the behaviour of the human toward the piglet was adapted to the reaction  
846 of each animal and included four steps: (1), the handler hold out the hand towards the animal; (2) if  
847 the piglet did not move away, the handler tried to touch it; (3) if the piglet accepted being touched,  
848 the handler softly stroked it along the body with the palm of her hand; and (4) once it accepted being

849 stroked, the handler scratched it along the body with her fingers. Scratching consisted in rubbing the  
850 skin of the piglets with the finger tips and applying more pressure than stroking. No specific body  
851 part of the piglets was more considered than another. Two experimenters ('AV' and 'AH') performed  
852 these sessions (both women, both between 1.70-1.73 m tall, with a balanced number of pens  
853 attributed to each of them). The experimenters wore the same blue overalls and green boots each  
854 time they interacted with the piglets. The experimenters tried to imitate each others behaviours  
855 (remote video monitoring) to decrease variability.

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

### 860 Conditioning: sessions of additional positive contacts with 861 (un)familiar human

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

867 Briefly, all piglets were individually trained to learn to associate two different stimuli with the  
868 arrival of two different (pseudo)-social partners: either two pen mates (partner = Conspecifics) or a  
869 familiar human (partner = Human). When entering the room, the piglets and the partner(s) would  
870 remain in the room for 2 min. Specifically, when the human was the partner, the human entered, sat  
871 on a bucket and positively interacted with the piglet for 2 min, in the same manner as additional  
872 contacts was provided to the H+ piglets during the previous period (see above section) (figure 1).  
873 Therefore, at the beginning of the conditioning, H+ piglets were already familiar with the human and  
874 procedure, whereas H piglets were unfamiliar with the human. During the conditioning, the same  
875 sessions occurred in both treatment groups (H and H+). After the conditioning, all piglets were  
876 familiar with the human, but treatment groups had a different time of exposure to them. Sessions of  
877 reunions with social partners were not studied here because they were part of an analysis on vocal  
878 expression of positive anticipation reported earlier (Villain, Hazard, et al., 2020).

879 For every second trial, the 2 min reunions with the human were analysed by the same person: trials  
880 number 2, 4, 6, 8, 10 and 11 (see behavioural analyses section).

## 881 Standard Isolation/Reunion Tests with a static and silent human

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

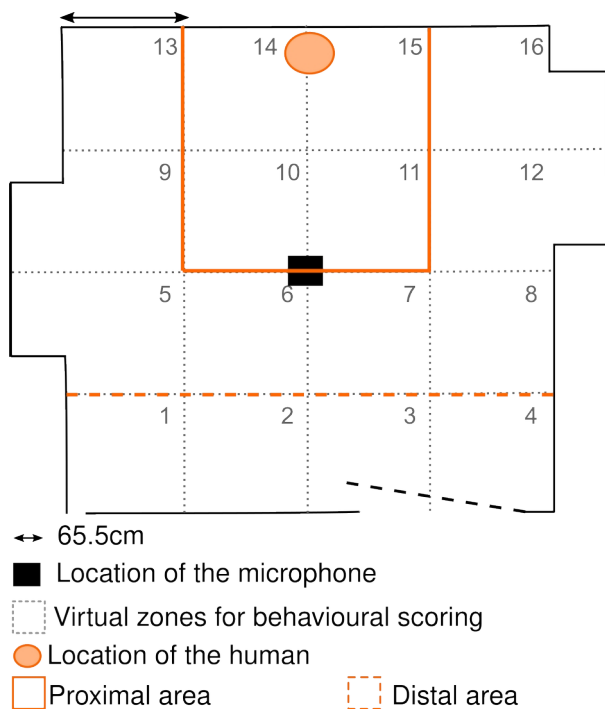


Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1, 2, 3, 4) were defined, using the location of the human as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

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

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

Table 1: Ethogram.

Behaviour	Description
No. zones crossed <sup>1,2</sup>	The number of times the piglet crossed a virtual zone
No. approaches H <sup>1</sup>	Number of times the piglets entered the proximal area
Time watching H <sup>1,2</sup>	The amount of time the piglet spent watching the human
Latency to contact H <sup>1,2</sup>	The latency to the first contact of the human by the piglet
No. looks toward H <sup>1,2</sup>	The number of times the piglet turned its head toward the human
No. looks at walls and doors <sup>1</sup>	The number of times the piglet looked at walls and doors
Time watching walls and doors <sup>1</sup>	The amount of times the piglet watched walls and doors
Time in proximal area <sup>1,2</sup>	The amount of time the piglet spent in the proximal area
Time in distal area <sup>1,2</sup>	The amount of time the piglet spent in the distal area
Time in contact H <sup>1,2</sup>	The amount of time the piglet investigated the human
Time investigating floor <sup>1,2</sup>	The amount of time the piglet investigated the floor
No. contacts H <sup>2</sup>	Number of times the piglet was in contact with the human (initiated by the piglet or the human)

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

898

## 899 Acoustic monitoring and analyses

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

912 A spectro-temporal analysis was performed with custom-written codes using the Seewave R  
 913 package (Sueur et al., 2008) implemented in R (R Core Team, 2022). We first studied the spectral  
 914 properties of the remaining background noise of the experimental room (electric noises and  
 915 remaining low frequency noises from the rest of the building), using 20 examples of 0.5 second  
 916 fragments and compared it with the general frequency range of the grunts. To avoid measuring  
 917 masking effect of the background noise, grunts were filtered using a 0.2-8 kHz bandpass filtering  
 918 (‘fir’ function). As a consequence, all results presented in this study are on a 0.2-8 kHz frequency  
 919 range, and no conclusions on frequency components of grunts below this 200 Hz threshold are drawn

920 here. Several acoustic parameters were then extracted from each grunt. To measure grunt duration, a  
 921 5% to maximal amplitude threshold was used ('timer' function). After normalisation, the following  
 922 spectral parameters were calculated using the 'specprop' function (FFT with Hamming window,  
 923 window length = 512, overlap = 50%): mean (Q50), first (Q25) and third (Q75) quartiles,  
 924 interquartile range (IQR), centroid and standard deviation (all in Hz). The grunt dominant frequency  
 925 (in kHz) was also calculated ('dfreq', 50% overlapping FFTs, window length = 512), which is the  
 926 mean over the grunt duration of the frequencies of highest energy of each window. Frequency peaks  
 927 were detected and the minimal and maximal peaks were kept as descriptors ('fpeaks' function,  
 928 window length = 512, peak detection threshold = 10% of the normalized amplitude). Measures of  
 929 noisiness and entropy of the grunts were assessed using: Shannon entropy (sh), Spectral Flatness  
 930 (Wiener entropy, sfm) and Entropy (H) [combining both Shannon and Temporal envelop entropy,  
 931 length = 512, Hilbert envelop). Two vocal scores were used: the logarithm of grunt duration and a  
 932 built-in spectral vocal score with all spectral parameters (see below). A table describing mean and  
 933 range of variation of each acoustic parameter in the relevant contexts of the study is available in the  
 934 supplementary material (table S4).

## 935 Statistical analyses

### 936 ***Behavioural and vocal response scores***

937 All measures extracted from videos or sound analysis are named parameters throughout the text.  
 938 The symmetrical distribution of parameters (behavioural on the one hand and acoustic on the other  
 939 hand) was visually inspected, and linear transformations were computed when necessary to reach  
 940 symmetrical distribution (see tables 2, 3, 4). When this criteria was reached, Principal Component  
 941 Analyses (PCA, one for the behavioural analysis and one for the spectral acoustic analysis) were  
 942 performed using several parameters to build scores ['dudi.pca' function from 'ade4' R package  
 943 (Dray & Dufour, 2007) and 'inertia.dudi' function to extract the loadings]. These scores were then  
 944 used as statistical variables. Indeed, PCAs are generally used to reduce the number of variables  
 945 included in statistical models. It also generates quantifiable global descriptors of behaviours or  
 946 acoustic structure, since correlated parameters usually load on the same PC (McGregor, 1992). All  
 947 PCs having an eigenvalue above one were kept and constituted response scores of behavioural  
 948 ('ReuPCs' and 'CondPCs' in table 2 and 3 respectively) and vocal ('VocPCs', table 4) parameters.  
 949 Only the duration of grunts was kept separated from the spectral parameters to keep it as a temporal  
 950 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
No. of zones crossed (sqrt)	24.177	<b>-55.843</b>	-0.435
No. approaches H (ln)	<b>47.748</b>	-30.163	0.578
Time watching H (ln)	<b>-52.914</b>	-7.422	25.585
Latency to contact H (ln)	<b>-64.232</b>	-0.464	1.688
No. looks toward H (sqrt)	-7.787	<b>-43.721</b>	31.633
Time watching room	-32.048	-13.581	-6.238
No. looks at walls and doors (sqrt)	3.524	<b>-72.408</b>	-2.027
Time in proximal area (sqrt)	<b>69.96</b>	-0.156	9.584
Time in distal area (sqrt)	<b>-46.416</b>	-12.437	-1.215
Time in contact H (sqrt)	<b>61.041</b>	3.586	24.183
Time spent investigating floor	11.868	-7.503	<b>-42.265</b>

951

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)	<b>80.23</b>	2.542	-0.112
Time in distal area (ln)	-33.826	8.547	30.789
Number of contacts H (ln)	<b>78.55</b>	6.476	2.288
Time in contact H (ln)	<b>86.625</b>	0.715	-0.369
No. looks toward H (ln)	-2	<b>79.898</b>	-0.745
Time watching H (ln)	-6.757	<b>65.67</b>	-10.325
No. of zones crossed (sqrt)	0.129	33.599	<b>48.457</b>
Time spent investigating floor	0.006	<b>-49.286</b>	14.205
Latency to contact H	<b>-81.01</b>	-0.248	-2.83

952

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 <sup>1</sup>	-13.558	<b>53.557</b>	2.220
Min frequency peak <sup>1</sup> (ln)	-0.349	<b>58.758</b>	24.236
Max frequency peak <sup>1</sup>	<b>-43.023</b>	8.760	-9.537
Mode <sup>2</sup> (ln)	-0.522	<b>66.248</b>	19.268
Mean <sup>2</sup> (ln)	<b>-95.092</b>	-2.295	2.028
Q50 <sup>2</sup> (ln)	<b>-85.278</b>	0.280	-0.093
Q25 <sup>2</sup> (ln)	<b>-52.360</b>	19.327	0.985
Q75 <sup>2</sup> (sqrt)	<b>-88.925</b>	-4.645	2.309
Centroid <sup>2</sup> (ln)	<b>-95.092</b>	-2.295	2.028
Sd <sup>2</sup>	<b>-64.484</b>	-11.303	7.680
IQR <sup>2</sup>	<b>-87.981</b>	-5.851	2.640
Sfm <sup>3</sup> (sqrt)	<b>-94.344</b>	-3.189	0.962
Sh <sup>3</sup> (sqrt)	<b>-96.087</b>	-0.785	-0.175
H <sup>3</sup>	<b>-88.205</b>	-1.059	-1.063
Skewness <sup>4</sup>	28.032	-18.010	<b>48.652</b>
Kurtosis <sup>4</sup>	22.973	-16.241	<b>50.615</b>

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

## 953 *Statistical models*

954 All statistics were carried out on R (R Core Team, 2022). Linear mixed effect models [*lmer*  
955 function, *lme4* R package (Bates et al., 2014)] were built when tested variables were linear  
956 (behavioural and vocal scores, grunt duration) and one binomial generalized mixed effect model was  
957 built for binary parameters (occurrence of missed contacts initiated by human during the  
958 conditioning). The following subsections describe how models were built for each type of tests. In all  
959 models described below, the identity of the replicate ('1' or '2') was used as an interacting fixed

960 factor, since the experiment was run in two identical replicates on two independent groups. The  
 961 identity of the human ('AH' or 'AV') was used as interacting fixed factor in all models described  
 962 below, since two experimenters were involved in the positive handling at weaning and in the session  
 963 of additional positive contacts of the conditioning (but always the same human was attributed to a  
 964 given piglet). The piglet was used as random factor to take into account the within-subject design.  
 965 All explanatory variables used in the models and interactions between them were built in respect to  
 966 the experimental design and to allow biological interpretations. As a consequence, not all  
 967 interactions between all variables were made. They are fully explained in the subsequent sections.

### 968 **Isolation/Reunion tests**

969 The aim of this part was to test the effect of the positive handling at weaning treatment (H vs. H+  
 970 piglets) and additional human contacts during sessions of the conditioning on the piglet's reaction to  
 971 human presence. Since the same Isolation/Reunion test was repeated before and after the  
 972 conditioning, we used the variable 'Conditioning time' as a two level interacting factor ('before' or  
 973 'after' conditioning, referred as "Time" in the models) to test the effect of the conditioning. Piglets  
 974 spacial behaviour and proximity to the human was studied only during the reunion phase with the  
 975 human that followed the isolation phase. Model\_1 was computed:

```
976 Model_1 <- lmer (ReuPCs ~ Treatment*Time + Treatment*Replicate +
977 Treatment*HumanID + Time*Replicate + Time*HumanID + (1 | pigletID), data=
978 data_Behaviour_Reunion).
```

979 Concerning the analysis of vocal behaviour, the isolation phase represents a negative social context  
 980 for the piglets and may be used as a negative control when monitoring the effect of human presence  
 981 on vocal expression of emotional states (Villain, Lanthony, et al., 2020). So, the two phases of the  
 982 test were used to study the three way interaction between treatment (H vs.. H+), phase of the test  
 983 (isolation vs.. reunion) and time of the conditioning (before vs.. after). The following model\_2 was  
 984 computed:

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

988 To go further, only the reunion phase was kept and a proximity variable was added. Indeed, the  
 989 piglet could vocalise either when close to human or away from them and this spatial proximity was  
 990 demonstrated as an important factor of changes of vocal features (Villain et al. 2020b). Thus, a two  
 991 level proximity factor was built: either '1' when the piglet was in the proximal area (figure 1) or '0'  
 992 when it was elsewhere in the room. The following model\_3 was computed:



```

993 Model_3 <- lmer (Vocal response score ~ Treatment*Time*InProxArea +
994 Treatment*HumanID + InProxArea*HumanID + Treatment*Replicate +
995 InProxArea*Replicate + Time*Replicate + Time*HumanID + (1 | pigletID/Time), data
996 = data_Vocal_Reunion).

```

### 997 **Conditioning trials**

998 The aim was to study the evolution of human-piglet relationship over the conditioning [the variable  
999 ‘Trial number’, used as a continuous variable, referred as “Trial” in the models]. The effect of  
1000 treatment (positively handled at weaning H+ piglets or non handled H piglets) was tested as an  
1001 interacting factor with Trial. Trial was also used as a random slope to take into account individual  
1002 trajectories (Schielzeth and Forstmeier 2009). The following model\_4 was built to test the  
1003 behavioural response scores CondPCs (lmer) and the occurrence of missed contact initiated by the  
1004 human during a session (presence/absence, binomial model, glmer):

```

1005 Model_4 <- (g)lmer (CondPCs / Missed contact ~ Trial*Treatment + Trial*HumanID +
1006 Trial*Replicate + Treatment*Replicate + Treatment*humanID + (1+ Trial |
1007 pigletID), (family=Binomial), data= data_Behaviour_Conditioning).

```

1008 For the analysis of vocal response scores, similarly to the Isolation/Reunion test, the piglet could  
1009 vocalise either when close to the human or away from them. We thus added the proximity factor in  
1010 the analysis of vocal response variables. The following model\_5 was built :

```

1011 Model_5 <- lmer (VocPCs ~ Trial*Treatment*InProxArea+ Trial*HumanID +
1012 Trial*Replicate + Treatment*Replicate + Treatment*HumanID + HumanID*InProxArea +
1013 Replicate*InProxArea + (1+ Trial | pigletID), data= data_Vocal_Conditioning).

```

### 1014 **Model validation and statistical tests**

1015 All linear models were validated by visual inspection of the symmetrical and normal distribution of  
1016 the residuals. Anovas (‘car’ R package (Fox & Weisberg, 2011)) were computed on models to test  
1017 for significant effects of explanatory variables. Following the Anova, when interactions were found  
1018 significant, post hoc test were run on model interactions, correcting for multiple testing with Tukey  
1019 contrasts (‘emmeans’ or ‘lrends’ functions from ‘emmeans’ R package (Lenth, 2016), for  
1020 categorical or continuous variables respectively). Considering the conditioning time (before or after  
1021 conditioning), when involved in a significant three-way interaction, this factor was fixed to allow  
1022 pairwise comparison within each time period as it was not considered relevant to assess the effect of  
1023 time only. Results of the Anova, model estimates and pairwise post hoc comparisons are reported in  
1024 the supplementary material (tables S1 and S2 for tests, table S3 for model estimates).

1025

1026 **Results**1027 Effect of positive handling at weaning and conditioning on piglets'  
1028 reaction to human presence (Isolation/Reunion tests)1029 ***Piglets that were not handled at weaning express a similar behavioural proximity***  
1030 ***to a human after a positive conditioning as the positively handled ones.***

1031

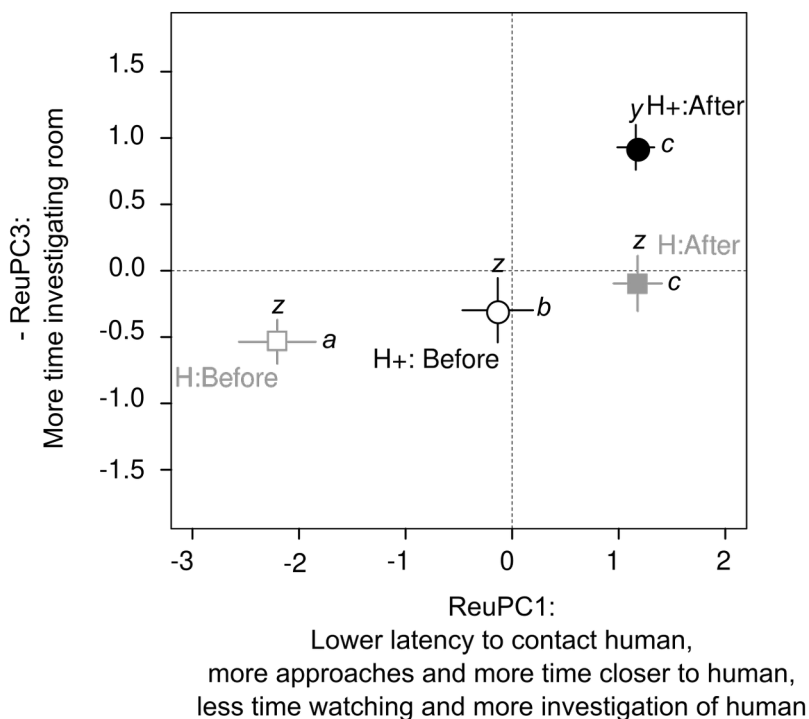


Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean  $\pm$  SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey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

1032 The interaction between the treatment (positively handled piglets at weaning (H+) or not (H) and  
1033 the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3  
1034 ( $\chi^2_1 = 28.0$ ,  $p < 0.001$ , and  $\chi^2_1 = 3.7$ ,  $p = 0.05$  respectively, figure 2) but not for ReuPC2 ( $\chi^2_1 <$   
1035  $0.001$ ,  $p = 0.99$ , supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was higher  
1036 after the conditioning than before (H: after – before, t.ratio = 12.1,  $p < 0.001$ , H+: after – before  
1037 t.ratio = 11.0,  $p < 0.001$ ) and that before the conditioning, piglets that were positively handled at  
1038 weaning had significantly higher ReuPC1 than non handled piglets (Before, H – H+: t.ratio = -2.1,  $p$   
1039  $< 0.001$ ), but not after (After, H – H+: t.ratio = 0.02,  $p = 1.0$ ). According to the loadings, this means

1040 that piglets that were positively handled at weaning had a lower latency to contact the human,  
 1041 approached them more often and spent more time close to and investigating the human (ReuPC1)  
 1042 than non handled piglets, before the conditioning. This score increased after the conditioning and no  
 1043 evidence of a difference between treatments after the conditioning was found (figure 2). Post hoc  
 1044 tests on ReuPC3 showed a significant effect of the conditioning time only in piglets that were  
 1045 positively handled at weaning (H+: after – before, t.ratio = 5.2,  $p < 0.001$ , H: after – before, t.ratio =  
 1046 2.6,  $p = 0.06$ ). No difference in ReuPC3 was found between treatments before the conditioning  
 1047 (Before: H – H+, t.ratio = -0.75,  $p = 0.87$ ), whereas positively handled piglets had a higher -ReuPC3  
 1048 after the conditioning than before (After : H – H+, t.ratio = -3.2,  $p = 0.009$ ). According to the  
 1049 loadings, this means that after the conditioning, piglets that were positively handled at weaning  
 1050 expressed more investigation of the room after the conditioning than non handled piglets. No  
 1051 evidence of any effect on ReuPC2 was found (table S2).

1052 ***Piglets positively handled at weaning produce shorter grunts even when no human***  
 1053 ***is present.***

1054 Using the isolation phase as a negative control we could compare the effect of the phase of the test  
 1055 (Isolation vs. Reunion with the human), taking into account the conditioning time (before or after the  
 1056 conditioning) and the treatment. No evidence of any effect of neither the three way interaction ( $\chi^2_1 <$   
 1057 0.62,  $p > 0.43$ ) nor two way interactions of interest was found (treatment: phase, conditioning  
 1058 time:phase, conditioning time: treatment interactions :  $\chi^2_1 < 3.5$ ,  $p > 0.06$ , table S2) in any of the  
 1059 scores.

1060 Regardless of the treatment, single effects of the phase of the test were significant for grunt  
 1061 duration and all AcPCs ( $\chi^2_1 > 6.6$ ,  $p < 0.01$ , table S1). During the reunion phase with the human,  
 1062 grunts were shorter (estimates of log(duration)[95% CI] : -1.32[-1.37;-1.26] vs. -1.06[-1.12;-1.00]),  
 1063 had a higher frequency range, higher bandwidth and a higher noise component (-VocPC1:  
 1064 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[-  
 1065 0.65;-0.28]) and their spectrum had a higher skewness and kurtosis (VocPC3: -0.25[-0.37;-0.14] vs. -  
 1066 0.11[-0.23;0.01] ), compared to the isolation phase.

1067 Regardless of the phase of the test, single effects of treatment were found for grunt duration and -  
 1068 VocPC3 ( $\chi^2_1 = 5.5$ ,  $p = 0.02$  and  $\chi^2_1 = 4.9$ ,  $p = 0.03$  respectively, table S2). Grunts produced by  
 1069 positively handled at weaning piglets were shorter (estimates of log(duration)[95% CI]: -1.25[-1.32;-  
 1070 1.19] vs. -1.12[-1.2;-1.1], table S3), and differed in -VocPC3 scores, describing the shape of the

1071 frequency spectrum (estimates of -VocPC3[95% CI]: -0.29[-0.43;-0.14] vs. -0.07[-0.22;0.08], table  
 1072 S3), than grunts produced by non handled piglets.

1073 **Positive handling and conditioning affect vocal reactivity to human proximity.**

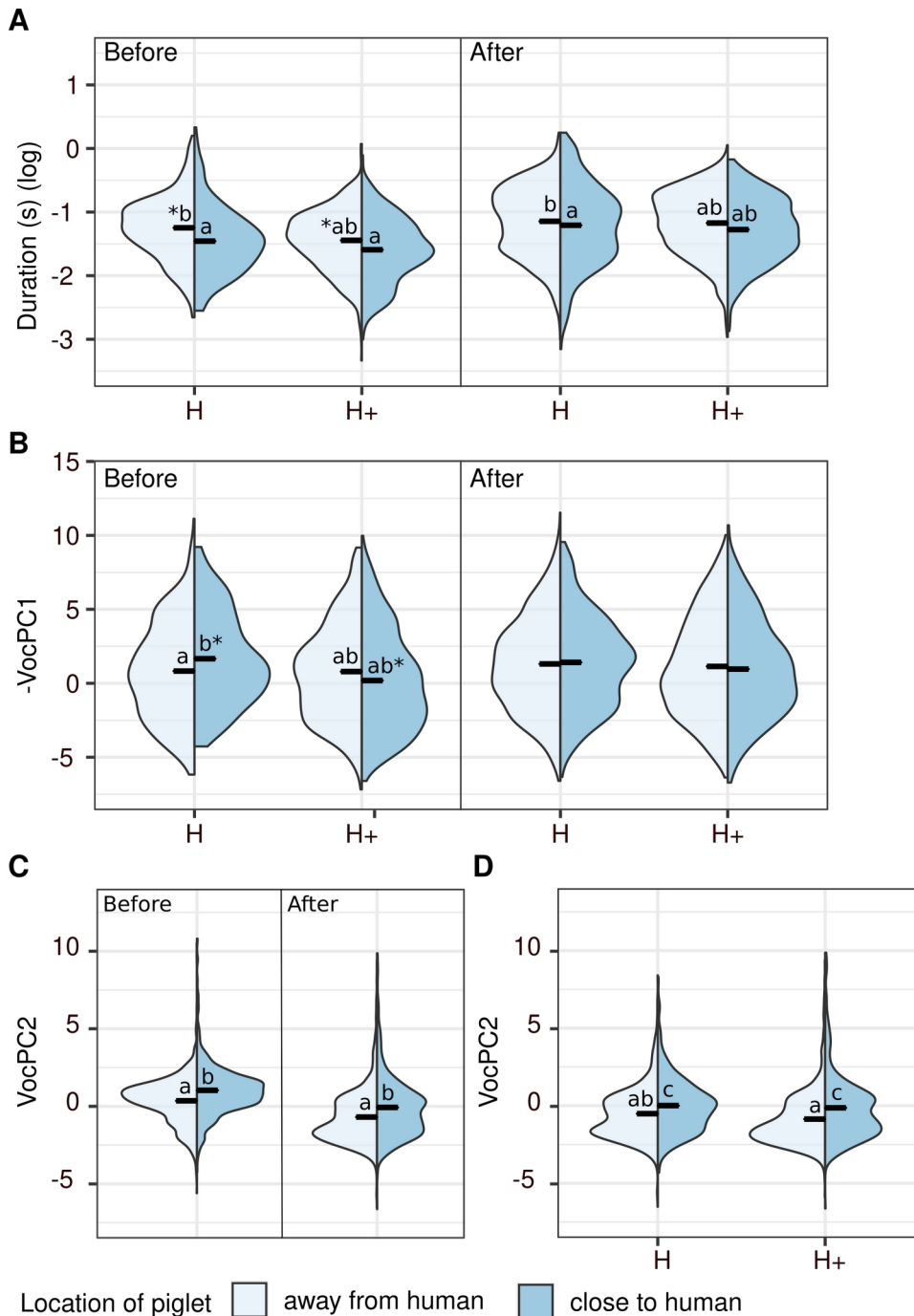


Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect of conditioning (before or after), treatment (H or H+) and location of the piglet relatively to the human (close: dark blue or away from them: light blue). Violin plots representing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

1074 During the 5 min reunion, the piglet was scored either as close to the human or away from them.  
1075 The three way interaction of the conditioning time, the treatment and the location was significant for  
1076 grunt duration, -VocPC1 and VocPC3 ( $\chi^2_1 > 4.9$ ,  $p < 0.03$ ). Post hoc tests revealed that grunts  
1077 produced closer to the human were shorter than the ones produced further away, but only in piglets  
1078 that were not positively handled at weaning, effect being stronger before the conditioning than after  
1079 it (H piglets: away – close,  $z.ratio = 6.3$ ,  $p < 0.001$  before and  $z.ratio = 4.1$   $p < 0.001$  after the  
1080 conditioning; H+ piglets: away – close  $z.ratio < 1.98$   $p > 0.19$ , figure 3A). -VocPC1 was higher, i.e.  
1081 grunts had a higher frequency range, bandwidth and were noisier when produced closer to the human  
1082 than further away, but only in non handled piglets and before the conditioning (H piglets: away –  
1083 close,  $z.ratio = -3.34$ ,  $p = 0.005$  before and  $z.ratio = -1.23$   $p = 0.61$  after the conditioning; H+ piglets:  
1084 away – close,  $z.ratio < 0.36$   $p > 0.21$ , figure 3B). For VocPC2, the three way interaction did not  
1085 reach significance ( $\chi^2_1 = 3.3$ ,  $p = 0.07$ ), so only subsequent two way interactions were considered  
1086 (post hoc tests on the three way interaction can be found in supplementary, tables S1 to S3). For  
1087 VocPC2, significant two way interactions were found between the conditioning time and the location  
1088 ( $\chi^2_1 = 10.3$ ,  $p = 0.001$ ) on the one hand, and between the location and the treatment ( $\chi^2_1 = 4.2$ ,  $p =$   
1089  $0.04$ ) on the other hand. Post hoc tests revealed that grunts produced closer to the human had a  
1090 higher VocPC2, meaning they had a higher pitch, effect being stronger before the conditioning than  
1091 after (before: away – close,  $z.ratio = -6.12$ ,  $p < 0.001$ ; after: away – close,  $z.ratio = -2.88$ ,  $p = 0.004$ ,  
1092 figure 3C). The increase in VocPC2 with the location was greater for non handled piglets than  
1093 positively handled piglets (H piglets: away – close,  $z.ratio = -5.54$ ,  $p < 0.001$ ; H+ piglets: away –  
1094 close,  $z.ratio = -3.82$ ,  $p = 0.001$ , figure 3D). The last two-way interaction of interest between the  
1095 conditioning time and the treatment did not reach significant level ( $\chi^2_1 = 0.80$ ,  $p = 0.37$ ). For  
1096 VocPC3, post hoc tests did not reach significant levels ( $|z.ratio| < 2.3$   $p > 0.09$  for any comparison) .

1097

1098 Emergence of positive perception of human (effect of additional  
 1099 positive contacts sessions over the conditioning)

1100 ***The conditioning increases behavioural proximity to the human in all piglets.***

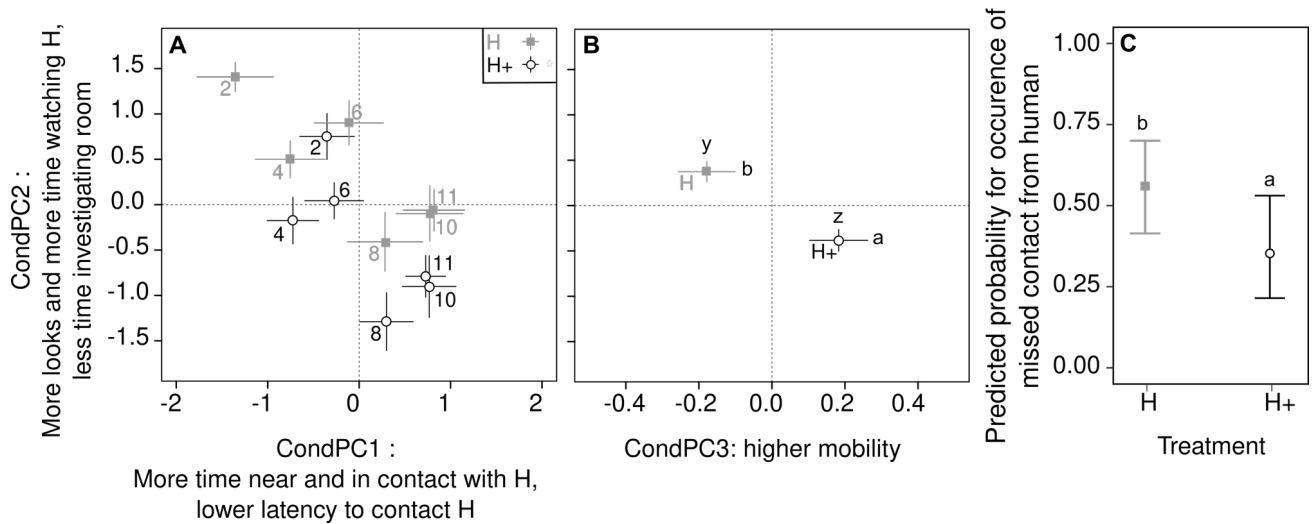


Figure 4: Behavioural variation of responses of piglets according to the sessions of additional positive contacts of the conditioning (A), and to the treatments (B, C). (A,B) Mean  $\pm$  SE per group. Numbers in (A) refers to the trial number of the conditioning. Higher CondPC1 and lower CondPC2 over time (single effect of trial number, A). Higher CondPC2 in H piglets than H+ piglets regardless of time (single effect of treatment, A). Higher CondPC3 and lower CondPC2 in H+ piglets than H piglets (single effect of treatment, B). (C) Mean estimates  $\pm$  95% confidence interval from generalized mixed effect model. Lower probability of occurrence of missed contact by the human in H+ than H piglets (single effect of treatment). Full statistical report is available as supplementary material (tables S1 et S2 for statistical tests, table S3 for model estimates).

1101 No evidence of any effect of the interaction between the treatment [positively handled piglets  
 1102 before the conditioning (H+) or not (H)] and the trial number was found for all behavioural scores  
 1103 (CondPC1, CondPC2 and CondPC3, table 3). Independently from the treatment, the higher the trial  
 1104 number the higher CondPC1 ( $\chi^2_1 = 59.3$ ,  $p < 0.001$ , slope estimate [95% confidence interval]: 0.20  
 1105 [0.15 : 0.25]) and the lower CondPC2 was ( $\chi^2_1 = 48.6$ ,  $p < 0.001$ , slope estimate: -0.17 [-0.22 : -  
 1106 0.12]). According to the loadings, over the conditioning, piglets decreased the latency to contact the  
 1107 human, made more contacts, spent more time in the proximal area and in contact with the human  
 1108 (condPC1), decreased the number of looks to the human, spent less time watching the human and more  
 1109 time investigating the room (CondPC2) (figure 4A). Independently from the trial number, positively  
 1110 handled piglets had a lower CondPC2 and a higher CondPC3 than the non handled ones ( $\chi^2_1 = 12.8$ ,  
 1111  $p < 0.001$  and  $\chi^2_1 = 7.0$ ,  $p = 0.008$  respectively), meaning that piglets that were positively handled at  
 1112 weaning expressed a fewer number of looks to the human, spent less time watching them and more  
 1113 time investigating the room (CondPC2) and crossed more virtual zone during the test (CondPC3)  
 1114 (figure 4B). The probability of having at least one missed contact by the human during a session was

1115 lower for positively handled piglets than non handled ones ( $\chi^2_1 = 9.57$ ,  $p = 0.002$ , figure 4C), with no  
 1116 interaction with the trial number ( $\chi^2_1 = 0.22$ ,  $p = 0.064$ ).

1117 ***Additional positive contacts trigger shorter and higher pitch grunts in all piglets.***

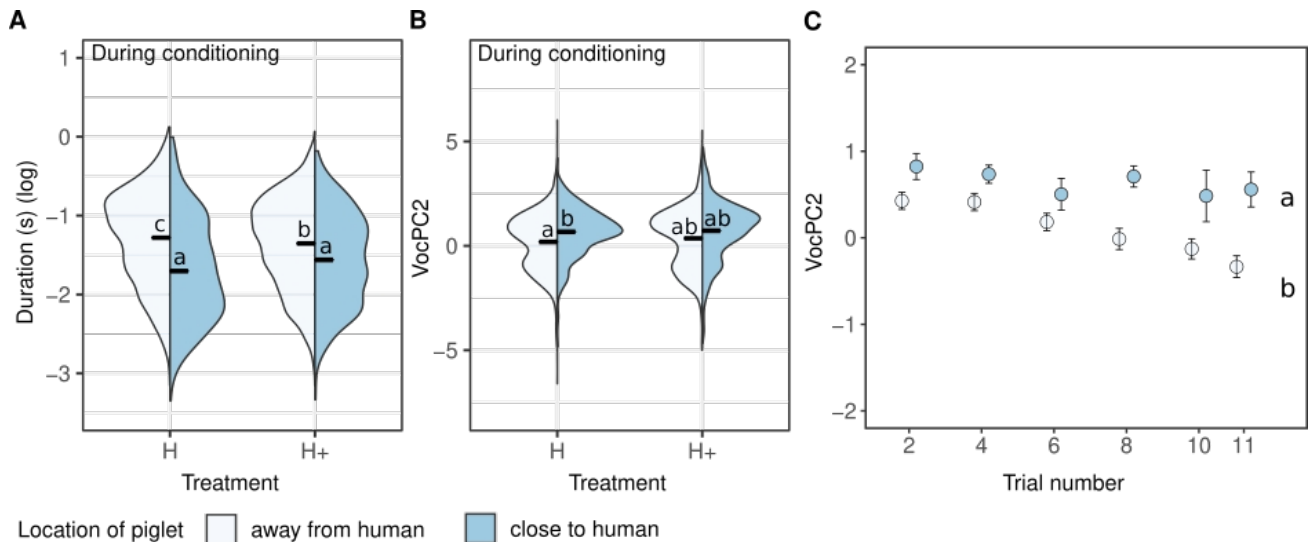


Figure 5: Vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human 'close': dark blue) or elsewhere in the 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).

1118

1119 During the sessions of additional positive contacts of the conditioning, the three-way interaction  
 1120 between the trial number, the treatment and the location was not significant for any of the vocal  
 1121 scores ( $\chi^2_1 < 0.18$ ,  $p > 0.67$ ), allowing the analysis of the two way interactions of interest. The  
 1122 interaction between treatment and the trial number was not significant for all vocal scores ( $\chi^2_1 < 2.5$   
 1123  $p > 0.11$ ). Grunt duration decreased over time and independently from the treatment (trial  
 1124 number:replicate interaction,  $\chi^2_1 < 5.3$   $p = 0.02$ , slope estimate  $-0.03[-0.04;-0.01]$  for the lower slope,  
 1125 table S1 and S3). However, independently from the trial number, grunt duration was lower when  
 1126 piglets were located close to the human and this effect was stronger in non handled piglets than  
 1127 positively handled piglets (treatment:location interaction:  $\chi^2_1 = 15.8$   $p < 0.001$ , away vs.. close, H  
 1128 piglets: z.ratio = 10.2  $p < 0.001$ , H+ piglets: z.ratio = 6.86  $p < 0.001$ , figure 5A). -VocPC1 and  
 1129 VocPC2 decreased over time but remained higher when piglets were located close to the human (trial  
 1130 number: location interaction,  $\chi^2_1 = 3.97$   $p = 0.05$  and  $\chi^2_1 = 6.1$   $p = 0.01$  respectively for -VocPC1 and  
 1131 VocPC2). According to the loadings, this means that the frequency range, bandwidth and noisiness  
 1132 of grunts (-VocPC1) as well as the pitch (VocPC2) decreased over the conditioning when piglets  
 1133 were located away from the human but remained high when piglets were close (slope comparison

1134 away – close, -VocPC1 : z.ratio = -1.80 p = 0.07, VocPC2 : z.ratio = -2.34 p = 0.02, figure 5C).  
 1135 Additionally, VocPC2 was higher when piglets were close to the human in non handled piglets  
 1136 (treatment:location interaction,  $\chi^2_1 = 7.6$  p = 0.005, pairwise comparisons away vs. close, in H:  
 1137 z.ratio = -4.9 p < 0.001 and in H+: z.ratio = -2.0 p = 0.21), meaning that non handled piglets  
 1138 produced higher pitched grunts when closer to the human (figure 5B).

## 1139 Impact of human identity on piglets behaviour and grunt structure

1140

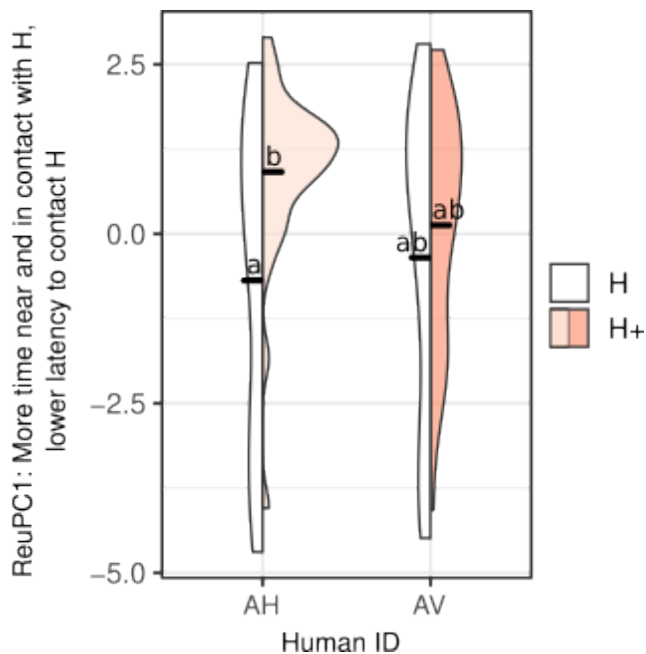


Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Values with no common letters differ significantly. Full statistical report is available as supplementary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

1141 Since half of the piglets had been assigned to one human experimenter and the other half to another  
 1142 one, the identity of the human was included in the model. This allowed to test interactions between  
 1143 the identity of the human and the treatment of positive handling at weaning on the one hand and the  
 1144 conditioning time on the other hand.

1145 During the reunions of the Isolation/Reunion test, the interaction between treatment and human  
 1146 identity was significant for the first behavioural proximity score (ReuPC1,  $\chi^2_1 = 6.01$ , p = 0.01) but  
 1147 not the others (ReuPC2 and ReuPC3 ( $\chi^2_1 < 1.98$ , p > 0.16, table S1). The effect of treatment on  
 1148 ReuPC1 was higher when piglets were handled by the human ‘AH’ (H vs. H+, AH: t.ratio = -4.77, p  
 1149 < 0.001, figure 6). When the human ‘AV’ handled the piglets, for which ReuPC1 scores exhibited  
 1150 intermediate values, treatment was not significant (AV, H vs. H+: t.ratio = -1.33, p = 0.56). These



1151 interacting effects of the human identity and treatment on behaviour were not found when  
1152 considering the reunions of the conditioning ( $\chi^2_1 < 1.32$ ,  $p > 0.25$  for all CondPCs, table S1).

1153 Interactions between the human identity and conditioning time were not significant, neither  
1154 considering the reunions of the Isolation/Reunion test (ReuPCs,  $\chi^2_1 < 0.642$ ,  $p > 0.42$ , tables S1),  
1155 neither the trial number during the session of additional positive contacts of the conditioning  
1156 (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
<b>Reunion of the Isolation/Reunion test</b>							
VocPC2	humanID	AH	0.154	-0.119	0.427	$\chi^2_1 =$ 4.94	P = 0.03
		AV	-0.292	-0.571	-0.012		
<b>Sessions of additional positive contacts of the conditioning</b>							
-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*I nProxArea	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		

1157

1158 Considering the vocal scores, no effect of human identity was found on VocPC1 during the  
1159 Isolation/Reunion tests but -VocPC1 was higher when the human 'AH' was in the room during the  
1160 reunion periods of the conditioning (table 5), meaning the frequency range and the bandwidth of the  
1161 grunt were higher when the human 'AH' interacted with the piglet compared to the human 'AV'.  
1162 VocPC2 was higher when the human 'AH' was in the room during the Isolation/Reunion tests (table  
1163 5), meaning that the pitch of grunts was higher and this effect was also found during the sessions of

1164 additional positive contacts of the conditioning in interaction with the location of the piglet ( $\chi^2_1 =$   
1165 11.9,  $p = 0.001$ ): VocPC2 increased when piglets were located close to the human but this increase  
1166 was significant only for the human 'AV' and not for 'AH' (table 5). VocPC3 was not different  
1167 between humans during the reunions of the Isolation/Reunion tests but, over the conditioning,  
1168 VocPC3 changed differently when piglets were handled by the human 'AH' or 'AV', as showed by  
1169 the significant interaction between trial number and human identity ( $\chi^2_1 = 8.0$ ,  $p = 0.005$ ): the  
1170 skewness and kurtosis of grunts decreased over the conditioning when 'AH' was interacting with the  
1171 piglets, but not 'AV' (see slope estimates, table 5). No evidence of any effect of human identity was  
1172 found on grunt duration neither during the Isolation/Reunion tests nor during the sessions of  
1173 additional positive contacts of the conditioning (table S1).

## 1174 **Discussion**

1175 In this study, familiarity to a human and human-animal interactions were experimentally modified  
1176 in weaned piglets to study the establishment of a positive HAR and test whether grunt structure could  
1177 reflect a positive HAR. A positive conditioning paradigm, using additional positive contacts from a  
1178 human as a reward, allowed to compare the behavioural changes over time in piglets previously  
1179 positively handled at weaning or not. Two types of sessions were studied: a standard  
1180 isolation/reunion tests with the human, carried out before and after conditioning, during which the  
1181 human remained silent and did not interact with the piglet, and sessions of the conditioning, during  
1182 which the human interacted with the piglets, providing additional positive contacts, as long as the  
1183 piglets stayed close to the seated human. Behavioural data were collected to describe the positive  
1184 HAR. Grunts produced during the tests and sessions were collected and their spectro-temporal  
1185 structure confronted to the behavioural data, with the hypothesis that vocalisation structure may  
1186 reflect the quality of HAR, though vocal markers of positive emotions. Firstly, the discussion will  
1187 focus on the behavioural validation of the establishment of a positive HAR. Secondly, behavioural  
1188 and vocal expression will be confronted to discuss grunt spectro-temporal structure as indicator of  
1189 the quality of HAR. Last, we will discuss perspectives regarding the effect of human identity on the  
1190 establishment of a positive HAR.

### 1191 Behavioural evidence of a rapid establishment of interest and 1192 proximity toward a human providing additional positive contacts

1193 The standard reunion test with the human before the conditioning showed first that the treatment of  
1194 positive handling at weaning succeeded in creating two different levels of human-piglet relationship

1195 (H and H+), as positively handled piglets expressed a higher attraction toward the human than non  
1196 handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al.,  
1197 2020). Second, this test showed that the conditioning increased the behavioural proximity toward the  
1198 human of both positively handled and non handled piglets so that non handled piglets expressed a  
1199 similar attraction toward the human as positively handled piglets. These results are in line with the  
1200 behavioural results of the sessions of additional positive contacts. The analysis of piglets' behaviour  
1201 every second sessions of the conditioning showed that, although positively handled and non handled  
1202 piglets started with different degree of proximity toward the human (trials 2 and 4, CondPC1), then,  
1203 over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human  
1204 (CondPC1) and avoided less the human when the latter attempted to interact with them. So it seems  
1205 that the conditioning process allowed non handled piglets to compensate the lack of positive  
1206 handling before the conditioning and develop a similar proximity toward the human. Two minute  
1207 daily sessions of additional positive contacts changed positively the perception of the human for the  
1208 piglets, and thus their willingness to interact with them. Since no evidence of any interaction  
1209 between time and treatment was found, no conclusion on differential developmental trajectories  
1210 between treatments can be drawn, but a parallel development of the human-piglet relationship in  
1211 both groups, when considering the proximity.

1212 Beside behavioural proximity, piglets that were positively handled at weaning expressed more  
1213 exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also  
1214 observed during the sessions of additional positive contacts of the conditioning: positive handled  
1215 piglets started with a higher score associated with investigation than non handled piglets (CondPC2)  
1216 and it held over the conditioning. Piglets that were positively handled at weaning also expressed a  
1217 higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an  
1218 expression of natural foraging and disinterest from human contact, which may be a sign of positive  
1219 welfare (Weerd & Day, 2009). In addition, this could also be interpreted in terms of attachment to  
1220 the human. Indeed, attachment to a human may facilitate exploration of novel environments or  
1221 objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may  
1222 provide an environment secure enough for the piglets to explore their environment in the presence of  
1223 the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al.,  
1224 2009).

1225 Overall, the behavioural monitoring showed that 2 min sessions of positive additional contacts per  
1226 day are sufficient to increase proximity to a human to similar levels as when piglets were previously

1227 familiarised for 2 weeks, even when piglets experienced social isolation. But it did not allow the non  
1228 handled piglets to express natural exploratory behaviours as the positively handled piglets. We  
1229 hypothesize a sequential establishment of a positive HAR over time: firstly with a decrease of  
1230 attentive state and an increase in proximity and accepted contacts, and secondly with a disinterest of  
1231 human contacts and the expression of natural foraging behaviour. The latter may require a higher  
1232 exposure time.

1233 In the next paragraph we discuss to what extent changes in grunt spectro-temporal structure may  
1234 reflect behavioural changes linked to the positive HAR over time.

### 1235 Links between vocal expression and positive HAR

#### 1236 *A positive HAR is reflected by shorter grunts in presence and absence of a human*

1237 The social isolation phase of the Isolation /Reunion test, before any human entered the room, was  
1238 associated with longer, lower pitched grunts with a downshifted frequency spectrum, whereas the  
1239 reunion with a static human changed grunts structure to shorter, higher pitched with an upshifted  
1240 frequency spectrum and this was observed in both handled and non handled piglets (H or H+) as well  
1241 as before and after the conditioning. In terms of emotional indicators, similar changes in acoustic  
1242 features of grunts were found in studies focusing on vocal markers of valence in pigs (Briefer et al.,  
1243 2019, 2022; Friel et al., 2019; Villain, Hazard, et al., 2020), meaning that the reunion with a human,  
1244 after a period of social isolation would be perceived as positive. However, this modulation of grunt  
1245 structure was observed regardless of piglet experience with the human. It is possible that the reunion  
1246 with an either neutral or familiar human, releasing piglets from total isolation could be perceived as  
1247 positive by the piglets, as suggested in previous studies (Villain, Lanthony, et al., 2020).

1248 In addition, and surprisingly, positively handled piglets produced shorter grunts than non handled  
1249 piglets regardless of human presence. This was previously shown in another context (anticipation of  
1250 (pseudo)social events independently from the type of partner) in the same groups of piglets (Villain,  
1251 Hazard, et al., 2020). This may show that the period of positive handling at weaning modulated vocal  
1252 expression in the long term, as this result was found both before and after the conditioning. On the  
1253 one hand, a positive HAR establishes through successive positive experiences (Rault et al. 2020)  
1254 and, on the other hand, HAR may have long term effects on behavioural expressions, as suggested by  
1255 Brajon et al. (2015) using cognitive bias tests. We can thus hypothesize this may also be reflected in  
1256 the way piglets vocalise, in general. In that case, we may have evidence of expression of another

1257 category of affect, moods, and not only emotional expression. Indeed, as suggested by Schnall  
1258 (2010), although emotions are short-term affects triggered by an external stimulus, moods, on the  
1259 other hand, may be experienced on a longer term and may not be attributable to a specific stimulus.  
1260 Although emotions and moods do not rely on the same time scale, they may interact with one  
1261 another, and more studies are needed to understand their effects on vocal expression.

### 1262 *A positive HAR affects vocal reactivity toward a static human*

1263 In a previous study, we showed that pigs vocalizing close to a human that previously had provided  
1264 repetitive additional positive contacts produced shorter and higher pitch grunts, compared to when  
1265 vocalizing away from the human (Villain, Lanthony, et al., 2020). Using the same type of test with  
1266 positively handled at weaning and non handled piglets, before or after conditioning sessions with  
1267 positive interactions, we can test the effect of positive handling on this modulation of grunt structure.  
1268 Similarly to the previous study, during the standard reunion test (no contact from the human), piglets  
1269 produced shorter and higher pitched grunts with an upshifted frequency spectrum when close to the  
1270 human. It has to be noted that this effect was 1) stronger in previously non handled piglets than  
1271 positively handled at weaning piglets and 2) stronger before the conditioning than after. In other  
1272 words, the more familiar with the human associated with positive handling, the less reactive to  
1273 human proximity.

1274 These results may be interpreted according to the behavioural results we described earlier (fig. 2).  
1275 We described that the proximity to the human was first increasing at the beginning of positive  
1276 handling experiences (see H piglets, before vs. after conditioning) before reaching a maximum (see  
1277 H vs. H+ piglets after conditioning) and that the most familiar piglets showed more exploratory  
1278 behaviours (H+ after conditioning). The acoustic results during the standard reunion mirror the  
1279 behavioural results from the same test. The least familiar piglets would vocally express the  
1280 exploration of a neutral and static human and, as the familiarity with the human increases, the human  
1281 may become part of their environment, explaining the lack of vocal reactivity when close to the static  
1282 human.

1283 In addition, we may also be facing ceiling effects in terms of vocal flexibility, which could also  
1284 partly explain these results. We showed that positively handled piglets generally produce shorter  
1285 grunts than non handled piglets, and that the shape of the frequency spectrum of these grunts was  
1286 different. So the structure of their calls, in general is different. According to the source-filter theory  
1287 of vocal production, vocal flexibility is constrained by the dimensions and functioning of the vocal

1288 apparatus (lung capacity, characteristics of the vocal folds, length and shape of the vocal tract, see  
1289 (Taylor & Reby, 2010) and (Titze & Martin, 1998)). It is possible that the positive HAR developed  
1290 by the positively handled piglets may have change their grunts structure to an extent that vocal  
1291 flexibility is no longer quantifiable in the experimental design of this study.

1292 ***Providing rewarding additional positive contacts triggers short and high pitched***  
1293 ***grunts***

1294 Contrary to the standard reunions with a static human, the human actively interacted with the  
1295 piglets during the sessions of the conditioning, providing contacts and producing speech as long as  
1296 the piglets remained close to the human. During these sessions and contrary to the standard reunions,  
1297 grunts produced close to the human were shorter and higher pitched, regardless of the trial number of  
1298 the conditioning and treatment. Although these effects were stronger in non handled piglets than  
1299 positively handled piglets, they remained over time. We describe here two types of vocal reaction to  
1300 human proximity, depending on the human behaviour. On the one hand, time decreased vocal  
1301 reactivity to human proximity during a standard reunion with a static human. On the other hand, no  
1302 evidence of a decrease in vocal reactivity to human proximity was found during sessions of  
1303 additional positive contacts. This would mean that positive interactions with piglets consistently  
1304 triggers the production of shorter and higher pitch grunts. These changes may be explained by the  
1305 expression of a higher arousal state experienced by the piglets while being positively handled.  
1306 Indeed, in the context of these sessions, the piglet could choose to approach and stay close to the  
1307 human, which will provide positive contacts systematically. So the piglet may anticipate to receive  
1308 positive contact and systematically being rewarded. When close to the human, observed changes in  
1309 frequency distribution of grunts (increased pitch and upshifted frequency spectrum) are known to be  
1310 markers of arousal (in the negative state in multiple mamalian species (Briefer, 2012, 2020) and pigs  
1311 (Linhart et al., 2015)). In addition, these spectral changes were also associated with shorter grunts.  
1312 Although the duration of grunts is associated with the valence of a situation, the duration may also be  
1313 an indicator of positive arousal. This hypothesis has to be taken precociously since no additional  
1314 control of arousal could be done in the present study.

1315 This working hypothesis may explain the decrease in vocal reactivity to human proximity observed  
1316 during the standard reunion test as the HAR becomes more positive. Indeed, before the sessions of  
1317 the conditioning, positively handled piglets were habituated to a human interacting positively when  
1318 present whereas non handled piglets were not, hence, during the first standard reunion test, when the  
1319 human is present but do not interact with the piglet, positively handled and non handled piglets may

1320 have diverging expectations regarding the presence of the static and silent human. As positively  
1321 handled piglets received positive contacts every time they were in the presence of the human, they  
1322 may have expected positive contacts when approaching and experienced an absence of reward during  
1323 the test. This has already been hypothesised in piglets deprived from human voice during interactions  
1324 after a period of habituation to it (Bensoussan et al. 2020). On the contrary, piglets that were not  
1325 positively handled at weaning never experienced additional positive contacts and being close to a  
1326 human, having the possibility to investigate them may be some kind of reward after the period of  
1327 total isolation. After the conditioning, piglets from both treatments were conditioned to receive  
1328 additional positive contacts and both groups had experienced a first standard reunion test, so they  
1329 may both experience an absence of reward during the test, which may explain a lower reaction to  
1330 human proximity, and thus fewer changes on grunt spectro-temporal features.

1331 Last, we can raise the question whether changes in grunt structure in reaction to rewarding positive  
1332 contacts may also be associated with a specific human-pig communication. In other domestic  
1333 species, owner directed vocalisations has been shown (in cats, reviewed in (Turner, 2017); in dogs  
1334 (Gaunet et al., 2022)). In addition, studies have found similar socio-communicative behaviours  
1335 toward a human in socialized pigs and dogs (Gerencsér et al., 2019). Hence, we may profit from  
1336 testing the existence of human directed vocalisations in pigs, as consequences of their socio  
1337 communicative abilities.

### 1338 Effect of human identity on piglets' perception: perspectives on 1339 HAR

1340 We found that the identity of the human had effects on behavioural and vocal response scores.  
1341 Piglets that were handled by the human 'AH' had higher values of behavioural proximity (ReuPC1)  
1342 than piglets handled by the human 'AV' during reunion test after a period of isolation. This effect  
1343 was not found during conditioning sessions. The effect of the human did not interact with the  
1344 conditioning time, leading to the conclusion that the difference between the two experimenters may  
1345 have established during the period of positive handling at weaning, prior to the conditioning.  
1346 Additionally, when the human 'AH' was in the room, piglets produced grunts with a more upshifted  
1347 frequency spectrum and a higher pitch than when the human 'AV' was in the room. If upshifted  
1348 grunts may be a indicator of positive higher arousal, then we may conclude that 'AH' was more  
1349 likely to trigger higher positive states than 'AV'. Interestingly, the human identity and the spatial  
1350 proximity had different effects on piglets grunts during sessions of additional positive contacts but  
1351 not when the human was static during the standard reunion test. Hence, it is possible that the way one

1352 human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering  
1353 positive emotions and thus modifications of grunt structure. Several evidence exists in the literature  
1354 that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al.,  
1355 2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We  
1356 may question the efficiency of different human features to generate a positive HAR. In our study,  
1357 both humans that interacted with the piglets wear exactly the same clothes and standardized their  
1358 tactile interactions toward the piglets before starting the study, and agreed on the rhythm and types of  
1359 sounds (words, intonation) to use, to minimise generating variability although no systematic controls  
1360 of the human behaviour or spectral feature of voices were performed here. It thus remains unclear  
1361 whether experimenters interacted differently or if they were initially perceived differently by piglets.  
1362 Our results show that the identity of the human may modulate piglet proximity and vocal behaviour  
1363 but the design of this experiment does not allow to find the causes of these observations (behaviour,  
1364 voice characteristics, or even odour profile). Thus, more studies of human features that are most  
1365 likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In  
1366 addition, studying human-piglet relationship in a more systematic way, as in other domestic species,  
1367 for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech  
1368 (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies  
1369 of interspecific relationships. However, the influence of human identity did not modify the general  
1370 outcomes of our study, but only decreased some effects, suggesting that this variability does not  
1371 modify the main results, but should be considered in future studies.

1372

1373 To conclude, we showed that degrees of familiarity toward a human could be reflected in the way  
1374 piglets vocalise in their presence, and out of it. We also showed that the spatial proximity toward a  
1375 human providing additional care could change the acoustic structure of piglet grunts. These changes  
1376 are likely to be linked to positive and more intense emotional states than when piglets are further  
1377 away from the human. However, it is still unclear whether the changes in grunt structure could also  
1378 be linked to human-animal communication and more studies are needed to determine it. We did also  
1379 show that the identity of the human may be of importance, and may generate vocal changes during  
1380 additional positive contacts that were not associated with changes in behaviour of the human. More  
1381 systematic studies of human behaviour along with pig behaviour during the human-animal  
1382 interactions would be needed to have a better understanding of the evolution of HAR, especially  
1383 interactive interspecific communication as well as providing new procedures to promote positive



1384 welfare. We suggest that analysing vocalisations structure may be a good tool to assess the quality of  
1385 human-pig relationship and help monitor the establishment of a positive HAR.

## 1386 **Authors contributions**

1387 Conceived and designed the experiment (A.V., C.T., C.N.). Performed the experiment (A.V., C.G.).  
1388 Collection and processing of the acoustic and behavioural data (A.V., C.G.). Statistical analyses  
1389 (A.V.). Contributed to the writing of the manuscript (A.V., C.T., C.N.).

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## 1397 **Data availability**

1398 The datasets used for the study are available at (Villain et al., 2022). The folder contains all datasets  
1399 and a readme to match the type of analysis to the proper dataset. We have made sure to report in the  
1400 main text of the article which R libraries and which functions in these libraries we used. All formulas  
1401 of the statistical models are explicit in the text to facilitate transfer of information and replicate the  
1402 analysis. All libraries are open source as well.

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