



31 produced shorter grunts than untamed piglets. However untamed piglets expressed more flexibility in  
32 call structure when vocalising close to a human, with a decrease of grunt duration and an increase of  
33 pitch, frequency range and noisiness in their grunt. This differential effect of proximity in tamed and  
34 untamed piglets was attenuated after the conditioning during a standard reunion with a static human  
35 but remained over time when the human was providing additional positive contacts. Results suggest  
36 that first, changes in vocal structure are consistent with indicators of positive states in the presence of  
37 a human. Second, increasing familiarity and proximity between a human and a piglet may induce  
38 changes in the acoustic structure of their grunts. Third, a carrying human trigger more changes in  
39 vocalisation structure than by their presence only. We show that vocalisation structure may allow to  
40 assess the quality of human-pig relationship.

## 41 **Introduction**

42 The long process of domestication has conducted to shape physiology and morphology of domestic  
43 animal species, but also their behaviour. It notably has shaped interspecific interactions between  
44 human and non human animals, by improving animals' capacity to use human signals to adapt their  
45 behaviour, by decreasing fearfulness toward humans and increasing attention toward humans  
46 (Mignon-Grasteau et al. 2005). Domestic species form particular relationship with humans. In farms,  
47 this relationship is important for animal welfare. Therefore understanding the mechanisms of  
48 emergence and maintenance of a positive human-animal relationship (HAR), by studying human-  
49 animal interactions and their consequences would have applications for welfare (Rault et al. 2020).  
50 Animal welfare conveys three major aspects: the ability of an animal to control its mental and  
51 physiological stability (Broom 2011), the decrease of negatively perceived as well as the increase of  
52 positively perceived contexts and species-specific behaviors (Peterson, Simonsen, and Lawson 1995;  
53 Weerd and Day 2009). A positive HAR is thought to be established through repeated positive  
54 interactions between the human and the non human animal, accumulating positive experiences  
55 though positive associative learning, modifying cognitive biases, generating expectations from the  
56 non human-animal toward the human and can be appreciated through behavioural and physiological  
57 measures as well as the expression of positive emotions (reviewed in (Rault et al. 2020)). Several  
58 behavioural measures may help to define a positive HAR such as: short latency to approach and  
59 spatial proximity (Schmied et al. 2008; Boivin, Tournadre, and Le Neindre 2000), body postures  
60 (Villain et al. 2020b) or play behaviour (Jerolmack 2009). Contacts from a human such as stroking,  
61 may induce changes in body postures and exposition of body areas by the animal to the human,  
62 supposedly vulnerable [central neck area in cattle (Schmied et al. 2008), abdominal area in pigs (Rault

63 et al. 2019) . Such grooming solicitation may be markers of engagement, trust and motivation to  
64 interact with the human. In most cases, these behaviors are similar to those shown during  
65 intraspecific sociopositive interactions, although there are some interspecies specific behaviors (e.g.,  
66 dog vs. wolf (Gácsi et al. 2005)). Vocal behaviour may also help defining the quality of a HAR.  
67 First, vocalisations are known to carry markers of the emotional states in several bird and mammal  
68 species (Briefer 2012; 2020) and this has been extensively study in domestic farm animals reviewed  
69 in (Laurijs et al. 2021). Second, some vocalisations have been associated to positive interactions with  
70 or care soliciting from humans, for example the cat – human communication : purring is thought to  
71 be derived from mother pup communication during nursing and is observed associated to care  
72 sollicitation from humans ; meowing, which is not observed during intra specific interactions is  
73 thought to emerge from associative learning during cat – human interactions (Brown and Bradshaw  
74 2014). This shows that HAR may elicit specific vocalisations from the non human animal toward the  
75 human.

76 In pigs, numerous evidence attest the possibility of a positive HAR. Animals may be tamed by  
77 humans providing regular additional positive contacts, leading to the expression of positive  
78 perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests  
79 showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et  
80 al. 2015a). Pigs may recognise a carying human compared to unfamiliar and adapt their behaviour  
81 accordingly (Brajon et al. 2015c). Pigs may be sensitive to human voice and respond accordingly  
82 (Bensoussan et al. 2019; 2020). Pigs vocalisations are diverse and linked to their emotional states,  
83 attested by the use of positive or negative vocal signals (Briefer et al. 2019; 2022; Tallet et al. 2013) .  
84 Indeed, even within a call type, spectro temporal changes are closely linked to the valence of a  
85 situation or the intensity of a perceived situation. For example, grunts, that are one of the most used  
86 vocal signals and in various situations is now known to be a flexible call: shorter grunts have being  
87 associated to positive situations (Briefer et al. 2019; 2022; Friel et al. 2019; Villain et al. 2020a), as  
88 well as higher formant and a lower fundamental frequency during positive situations (Briefer et al.  
89 2019; 2022; Villain et al. 2020a; Friel et al. 2019) . Grunt structure may also change according to the  
90 arousal of a situation, with a higher frequency range and a higher bandwidth when produced in a  
91 more intense positive situation (Linhart et al. 2015). In order to determine if vocalisations may be  
92 used as non invasive indicators of the quality of human-pig relationship by themselves, we tested  
93 whether they could encode the quality of the human-piglet relationship. We predicted that if grunts  
94 carry information on the quality of the human-piglets relationship, then 1. A period of positive  
95 interactions given by a human should modulate vocal quality of piglets when in presence of the

96 human, leading to grunts exhibiting markers of positive states (shorter grunts), 2. spatial proximity  
97 toward a human being should influence the vocal activity (higher pitched grunts as the arousal  
98 increases).

## 99 **Methods**

### 100 ***Ethical note***

101 The study was approved by the ethic committee CREEA and received the authorization no.  
102 APAFIS#17071-2018101016045373\_V3 from the French Ministry of Higher Education, Research  
103 and Innovation; and was in agreement with the French and European legislation regarding  
104 experiments on animals.

### 105 ***Subjects and housing conditions***

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

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

### 117 ***Experimental treatment : human additional contacts – taming period***

118 From day 28 to day 39 of life, piglets were separated into two treatment groups as follows:

- 119 • Untamed piglets, a group with minimal human contact, H group: Control piglets from 10  
120 rearing pens received the minimal amount of daily contact with a stockperson (a 1.70m tall  
121 male who did the feeding, cleaning and health checkups). The stockperson wore a dark green  
122 shirt and pants and brown shoes.
- 123 • Tamed piglets, a group receiving additional human contacts, H+ group: in addition to the  
124 daily care given by the same stockperson as for H group, piglets from the 10 other rearing  
125 pens received sessions of additional human contacts with one of the two experimenters (both  
126 women, both between 1.70-1.73 m tall, balanced number of pens attributed to each of them).  
127 The experimenters wore the same blue overalls and green boots each time they interacted

128 with the piglets. There were twenty-nine sessions of 10 minutes of interactions, from day 28  
129 (weaning) until day 39, occurring five days a week. Three sessions per day were performed  
130 (except on the day of weaning during which only two were done with a two-hour break in  
131 between). Each session took place in the rearing pen and the order of the interventions in the  
132 pens was balanced across days. The handling procedure, using gentle tactile contacts is  
133 described in supplementary material of Villain et al. (2020a) and was similar to Tallet et al.  
134 (2014).

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

136 Piglets were habituated to the test room for 10 minutes, by pen, two days before the start of the  
137 conditioning. The conditioning took place between day 42 and 62 after weaning and lasted twelve  
138 days, with two trials per day and at least three hours between trials on the same day. The  
139 experimental design of the conditioning is already published (Avelyne S. Villain et al. 2020a).  
140 Briefly, all piglets were individually trained to learn to associate two different stimuli with the arrival  
141 of two different (pseudo)-social partners: either two pen mates (partner = Conspecifics) or a familiar  
142 human (partner = Human). When entering the room, the piglets and the partner(s) would remain in  
143 the room for two minutes. Specifically, when the human entered, they sat on a bucket and positively  
144 interacted with the piglet, in the same manner as additional contacts was provided to the tamed  
145 piglets during the taming period (see above section) (figure 1). Hens, at the beginning of the  
146 conditioning phase, tamed piglets were already familiar with the human from the taming period,  
147 whereas untamed were unfamiliar with the human and only became familiar during the sessions of  
148 additional positive contacts of the conditioning. Since additional positive contacts occurred during  
149 the conditioning for both treatment groups (H and H+) the human could be considered as familiar for  
150 all piglets at the end of the conditioning, with a different degree of familiarity between the two  
151 groups. For every second trial, the two-minute reunions with the human were analysed : trials  
152 number 2, 4, 6, 8, 10 and 11.

### 153 ***Standard Isolation/Reunion Tests***

154 At 40 or 41 (before conditioning) and then 63 or 64 (after conditioning) and days of age, pigs were  
155 subjected to a standard Isolation/Reunion test in order to assess their perception of the human . The  
156 test consisted of two phases. The pig was brought individually in a trolley to the experimental room.  
157 It was left alone for five min, which defined the ‘Isolation’ phase. Then, the human entered the room,  
158 remained stand up for 30 secondes before they sat on a bucket, remaining silent and not moving for  
159 4.5 minutes (figure 1).

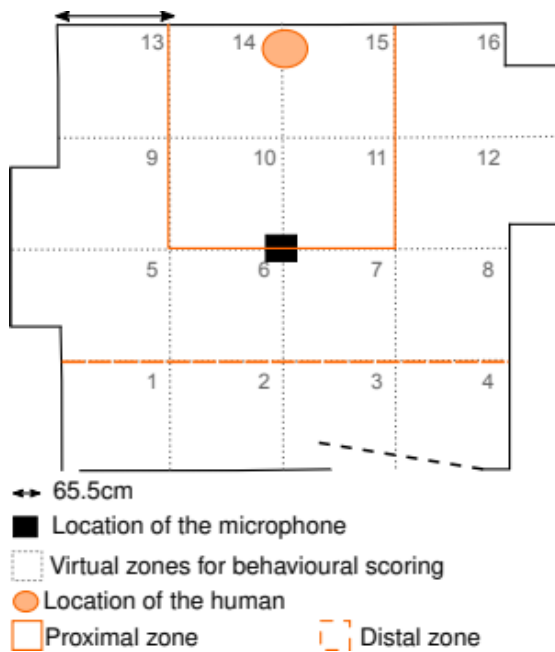


Figure 1: Design of the room used during the isolation/Reunion tests and the additional positive contact sessions of the conditioning. The room was split into 16 virtual zones and proximal and distal zones to the human regarding the position of the tested piglet.

## 160 **Behavioural monitoring and analyses**

161 Behaviours were monitored using a camera (Bosh, Box 960H-CDD) and annotated using *The*  
162 *Observer XT 14.0* (Noldus, The Netherlands) software. The square room was split into 16 virtual  
163 equally-dimensioned zones to assess the mobility and exploratory behaviour of the piglet. A  
164 proximal zone, around the human was defined by merging four zones, a distal zone was defined  
165 merging the four most distant zones from the human (figure 1).

166 The behaviours scored during the reunion of the Isolation/Reunion test and the sessions of additional  
167 positive contacts of the conditioning are available in table 1. Every time the shoulders of the piglet  
168 crossed a zone, a zone change was scored. Looks and watching behaviours were quantified as when  
169 the piglet turned its head toward the human or looked up at the room, and scored as point events, all  
170 over behaviours were scored as state events. Behavioural scores were then calculated to quantify  
171 global responses (see below).

172 Table 1: Ethogram.

Behaviour	Description
Nb zones crossed <sup>1,2</sup>	The number of times the piglet crossed a virtual zone



Nb approaches H <sup>1</sup>	Number of times the piglets entered the proximal zone
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
Nb looks toward H <sup>1,2</sup>	The number of times the piglet looked at the human
Nb looks other than H <sup>1</sup>	The number of times the piglet looked at other parts of the room
Time watching room <sup>1</sup>	The number of times the piglet watched other parts of the room than the human
Time in proximal zone <sup>1,2</sup>	The amount of time the piglet spent in the proximal zone
Time in distal zone <sup>1,2</sup>	The amount of time the piglet spent in the distal zone
Time in contact H <sup>1,2</sup>	The amount of time the piglet investigated the human
Time investigating room <sup>1,2</sup>	The amount of time the piglet investigated the room
Nb 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 for Reunion of Isolation/Reunion tests. <sup>2</sup>: Scored for reunion of conditioning sessions

173

#### 174 ***Acoustic monitoring and analyses***

175 Vocalisations were recorded with an AKG C314 microphone placed in the center of the room and  
176 one meter above the ground, connected to a Marantz MD661MK2 recorder. Vocalisations produced  
177 during each phase of the trial were manually annotated according to vocal type (grunt, squeak, bark,  
178 scream and mixed calls), after visual inspection of spectrograms on Praat software. Only grunts were  
179 analysed further as they were the most frequently expressed. A spectro-temporal analysis was  
180 performed with custom-written codes using the Seewave R package (Sueur, Aubin, and Simonis  
181 2008) implemented in R (R Core Team 2015) . We first studied the spectral properties of the  
182 remaining background noise of the experimental room (electric noises and remaining low frequency  
183 noises from the rest of the building), using 20 examples of 0.5 second fragments. Since the first  
184 quartile (Q25) of the normalized spectrum of the background noise was 250Hz and the grunts are  
185 low frequency vocalisations, we decided to remove all frequencies below 200Hz in order to focus on  
186 the most relevant frequencies, using a 0.2-8 kHz bandpass filtering ('fir' function). As a  
187 consequence, all results presented in this study are on a 0.2-8kHz frequency range, and no  
188 conclusions on possible frequency components of grunts below this 200Hz threshold can be drawn  
189 here. A standardised grunt was detected when the amplitude crossed a 5% amplitude threshold

190 ('timer' function) to measure the duration. After amplitude normalisation, the following spectral  
191 parameters were calculated using the 'specprop' function (FFT with Hamming window, window  
192 length = 512, overlap = 50%): mean, first (Q25) and third (Q75) quartiles, interquartile range (IQR),  
193 centroid and standard deviation (all in Hz). The grunt dominant frequency (in Hz) was also  
194 calculated ('dfreq', 50% overlapping FFTs, window length = 512), which is the mean over the grunt  
195 duration of the frequencies with the highest level of energy. Frequency peaks were detected and the  
196 minimal and maximal peaks were kept as descriptors ('fpeaks' function, window length = 512, peak  
197 detection threshold = 10% of the normalized amplitude) Parameters measuring noisiness and entropy  
198 of the grunts were: Shannon entropy (sh), Spectral Flatness (Wiener entropy, sfm) and Entropy (H)  
199 [combining both Shannon and Temporal envelop entropy, length = 512, Hilbert envelop). Two linear  
200 acoustic parameters were used: the logarithm of grunt duration and a built-in spectral acoustic score  
201 with all spectral parameters (see below). Table of acoustic data available in supplementary material  
202 (table S4).

### 203 ***Statistical analyses***

#### 204 Behavioural and vocal response scores

205 In order to take into account correlated parameters and reduce the number of variables tested as  
206 response variables in statistical models, all parameters having a symmetrical distributions were used in  
207 principal component analyses ('pca' function from 'ade4' R package (Dray and Dufour 2007, 4)).  
208 All PCs having an Eigen value above one were kept and constituted response scores for behavioural  
209 ('ReuPCs' and 'CondPCs' table 2 and 3 respectively) and vocal parameters ('AcPCs', table 4). Only  
210 the duration of grunts was kept separated from the spectral parameters to keep it as a temporal  
211 parameter.

212 *Table 2: Behavioural response score for the reunion phase of the Isolation/Reunion test. Percentage of explained*  
213 *variance and variable loadings of the principal component analysis. The first three PCs constituted three behavioural*  
214 *scores. Parameters that explain the most each PC are bolded ( $|loading| > 0.4$ ).*

	<b>ReuPC1</b>	<b>ReuPC2</b>	<b>ReuPC3</b>
Cumulative variance explained %	38.3	60.8	74
Nb zones crossed	24.177	<b>-55.843</b>	-0.435
Nb approaches H	<b>47.748</b>	-30.163	0.578
Time watching H	<b>-52.914</b>	-7.422	25.585
Latency to contact H	<b>-64.232</b>	-0.464	1.688
Nb looks toward H	-7.787	<b>-43.721</b>	31.633



Time watching room	-32.048	-13.581	-6.238
Nb looks other than H	3.524	<b>-72.408</b>	-2.027
Time in proximal zone	<b>69.96</b>	-0.156	9.584
Time in distal zone	<b>-46.416</b>	-12.437	-1.215
Time in contact H	<b>61.041</b>	3.586	24.183
Time spent investigating room	11.868	-7.503	<b>-42.265</b>

215

216 *Table 3: Behavioural response score for the reunions of additional positive contacts during the conditioning. Percentage*  
 217 *of explained variance and variable loadings of the principal component analysis. The first three PCs constituted three*  
 218 *behavioural scores. Parameters that explain the most each PC are bolded ( $|\text{loading}| > 0.4$ ).*

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

219

220 *Table 4: Variable loadings of the first three principal components (i.e having an Eigen value above one) following a*  
 221 *principal component analysis (pca function, ade4 R package) on all the grunts recorded in the entire dataset (including*  
 222 *both types of tests, N=17 546 grunts). The transformations used to reach symmetrical distribution before the PCA are*  
 223 *indicated in parenthesis. Parameters that explain the most each PC are bolded ( $|\text{loading}| > 0.4$ ).*

	<b>AcPC1</b>	<b>AcPC2</b>	<b>AcPC3</b>
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> (log)	-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> (log)	-0.522	<b>66.248</b>	19.268

Mean <sup>2</sup> (log)	<b>-95.092</b>	-2.295	2.028
Q50 <sup>2</sup> (log)	<b>-85.278</b>	0.280	-0.093
Q25 <sup>2</sup> (log)	<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> (log)	<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

## 224 Statistical models

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

### 236 *Isolation/Reunion tests*

237 The aim of this part was to test the effect of the taming treatment (H vs. H+ piglets) and additional  
238 human contacts during sessions of the conditioning on piglet's reaction to human presence. Since the

239 same Isolation/Reunion test was repeated before and after the conditioning, we used the parameter  
240 ‘Conditioning time’ as a two level interacting factor (‘before’ or ‘after’ the conditioning) to test the  
241 effect of the conditioning. Only the behaviour of the piglet during the 4.5min reunion with the sitted  
242 human was analysed.

```
243 Model 1 <- lmer ( Response variable ~ Treatment*Time + Treatment*Replicate +  
244 Treatment *Human + Time*Replicate + Time*Human + (1 | piglet ID), data=  
245 dataBehaviourReunion).
```

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

```
252 Model 2 <- lmer ( Vocal variable ~ Treatment * Phase * Time + Treatment *  
253 Human + Time * Human + Treatment * Replicate + Time * Replicate + (1 | piglet  
254 ID/time/Phase) , data= dataVocalIso + dataVocalReunion).
```

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

```
260 Model 3 <- lmer ( Vocal response parameter ~ Treatment * Time* In Prox. Zone +  
261 Treatment * Human + In Prox. Zone * Human + Treatment * Replicate + In Prox.  
262 Zone * Replicate + Time * Replicate + Time * Human + (1 | piglet ID/Time), data  
263 = dataVocalReunion).
```

### 264 *Conditioning trials*

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

```
272 Model 4 <- (g)lmer ( Behavioural Response variable ~ Trial * Treatment + Trial  
273 * Human + Trial * Replicate + Treatment * Replicate + Treatment * Human + (1+  
274 Trial | piglet ID), (family=Binomial), data= dataBehaviourConditioning).
```

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

```
278 Model 5 <- lmer (Vocal Response variable ~ Trial number * Treatment *  
279 InProximalZone+ Trial number * Human + Trial number * Replicate + Treatment *  
280 Replicate + Treatment * Human + Human * InProximalZone + Replicate *  
281 InProximalZone + (1+ Trial | piglet ID), data= dataVocalConditioning).
```

### 282 Model validation and statistical tests

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

291

292 **Results**

293 *Effet of the conditioning process on piglets' reaction to human presence*

294 *(Isolation/Reunion tests)*

295 *Untamed piglets express a similar behavioural proximity to a human after a positive*  
296 *conditioning than tamed piglets*

297

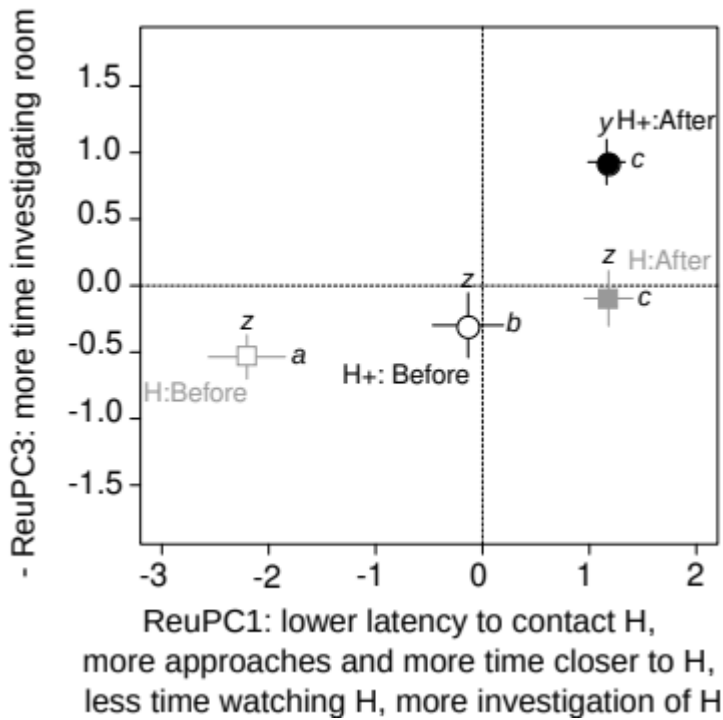


Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during a post isolation reunion test. Mean  $\pm$  SE per group is indicated, different letters indicated significantly different groups. Significant interaction between treatment (H : grey squares and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

299 The interaction between the treatment (piglets tamed before the conditioning (H+) or not (H)) and  
300 the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3  
301 ( $\chi^2_1 = 28.0$ ,  $p < 0.001$ , and  $\chi^2_1 = 3.7$ ,  $p = 0.05$  respectively, figure 2) but not for ReuPC2 ( $\chi^2_1 < 0.001$ ,  
302  $p = 0.99$ , supplementary table S1). Post hoc tests on ReuPC1 showed that ReuPC1 was higher after  
303 the conditioning than before (H: after – before, t.ratio = 12.1,  $p < 0.001$ , H+: after – before t.ratio =

304 11.0,  $p < 0.001$ ) and that before the conditioning, tamed piglets had significantly higher ReuPC1 than  
305 untamed piglets (Before, H – H+: t.ratio = -2.1,  $p < 0.001$ ), but not after (After, H – H+: t.ratio =  
306 0.02,  $p = 1.0$ ). According to the loadings, this means that tamed piglets had lower latency to contact  
307 the human, approached her more often and spent more time close to and investigating the human  
308 (ReuPC1) than untamed piglets before the conditioning. This score increased after the conditioning  
309 and no evidence of a difference between treatments after the conditioning was found (figure 2). Post  
310 hoc tests on ReuPC3 showed an significant effect of the conditioning time only in tamed piglets (H+:  
311 after – before, t.ratio = 5.2,  $p < 0.001$ , H: after – before, t.ratio = 2.6,  $p = 0.06$ ). No difference in  
312 ReuPC3 was found between treatments before the conditioning (Before: H – H+, t.ratio = -0.75,  $p =$   
313 0.87), whereas tamed piglets had a higher -ReuPC3 after the conditioning than before (After : H –  
314 H+, t.ratio = -3.2,  $p = 0.009$ ). According to the loadings, this means that tamed piglets expressed  
315 more investivation of the room after the conditioning than before. No evidence of any effect on  
316 ReuPC2 was found (table S2).

### 317 Taming decreases grunt duration even when no human is present with the piglet

318 Comparing the effect of the phase of the test (Isolation vs. Reunion with the human), taking into  
319 account the conditioning time and the treatment, no evidence of any effect of neither the three way  
320 interaction ( $\chi^2_1 < 0.62$ ,  $p > 0.43$ ) nor two way interactions of interest were found (treatment: phase,  
321 conditioning time:phase, conditioning time: treatment interactions :  $\chi^2_1 < 3.5$ ,  $p > 0.06$ , table S2) in  
322 any of the scores. However, grunts produced by tamed piglets were shorter than grunts produced by  
323 untamed piglets ( $\chi^2_1 = 5.5$ ,  $p = 0.02$ , estimates of log(duration)[95% confidence interval]: -1.25[-  
324 1.32;-1.19] and -1.12[-1.2;-1.1] respectively in tamed and untamed piglets, table S3). Single effects  
325 of the phase of the test were significant for grunt duration and all AcPCs ( $\chi^2_1 > 6.6$ ,  $p < 0.01$ , table  
326 S1). Grunts produced during the reunion phase with the human were shorter (estimates of  
327 log(duration) : -1.32[-1.37;-1.26] vs. -1.06[-1.12;-1.00]) and, according to the loadings, grunts  
328 produced during the reunion phase had a higher frequency range, higher bandwidth and noise  
329 component (-AcPC1: 0.78[0.48;1.08] vs. 0.34[0.03;0.66]), higher pitched (AcPC2: -0.18[-0.36;0.01]  
330 vs. -0.46[-0.65;-0.28] ) and their spectrum had a higher skewness and kurtosis (AcPC3: -0.25[-0.37;-  
331 0.14] vs. -0.11[-0.23;0.01] ), compared to the isolation phase.

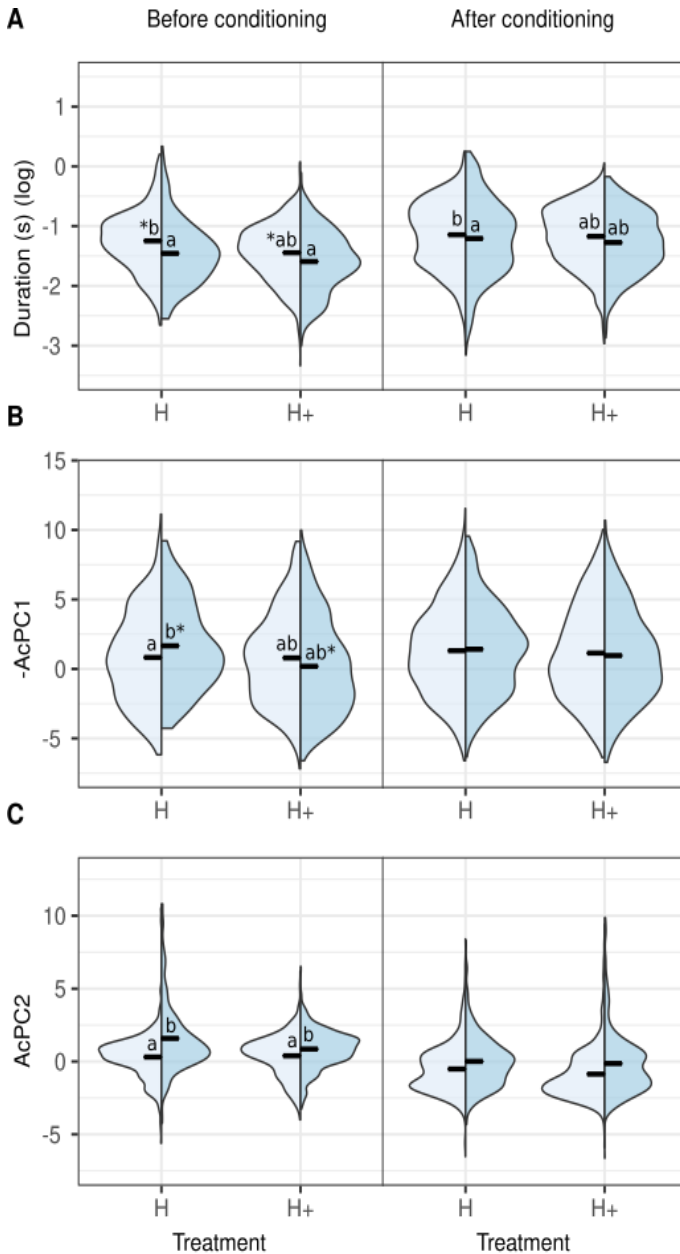
332

### 333 The conditioning process attenuates the effect of proximity on grunts vocal features in 334 untamed piglets

335

336





Location of piglet  away from human  close to human

*Figure 4: Acoustic structure of grunt during the reunions with a silent and static human (isolation/reunion test): effect of conditioning (before or after), treatment (H: light grey, H+: dark grey), 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 group. Results from the three way interaction between the treatment, the conditioning time and the location, on a subset of data considering only the reunion part of the test, the conditioning time was fixed to allow pairwise comparison of interacting location and treatment on grunt duration (A) and the first acoustic score (-AcPC1, B) and the second acoustic score (AcPC2, C). Letters represented significantly different groups ( $p < 0.05$ ) and 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).*

338 During the five-minute reunion, the piglet was scored either as close to the human or away from  
339 them. The three way interaction of the conditioning time, the treatment and the location was  
340 significant for grunt duration, -AcPC1 and AcPC3 ( $\chi^2_1 > 4.9$ ,  $p < 0.03$ ). Post hoc tests revealed that  
341 grunts produced closer to the human were shorter than the ones produced further away, but only in  
342 untamed piglets, effect being stronger before the conditioning than after it (H piglets : away – close,  
343 z.ratio = 6.3,  $p < 0.001$  before and z.ratio = 4.1  $p < 0.001$  after the conditioning, H+ piglets : away –  
344 close z.ratio < 1.98  $p > 0.19$ , figure 3A). -AcPC1 was higher, i.e grunts had a higher frequency  
345 range, bandwidth and were noisiness when produced closer to the human than further away, but only  
346 in untamed piglets and before the conditioning but not after (H piglets : away – close, z.ratio = -3.34,  
347  $p = 0.005$  before and z.ratio = -1.23  $p = 0.61$  after the conditioning, H+ piglets : away – close z.ratio  
348 < 0.36  $p > 0.21$ , figure 3B). For AcPC2, the three way interaction was close to reach significant level  
349 ( $\chi^2_1 = 3.3$ ,  $p = 0.07$ ), thus, for conservative purposes, the results of the posthoc tests of the three way  
350 interaction are presented (see two way subsequent interactions comparisons in supplementary tables  
351 S2 and S3 ). Before the conditioning, AcPC2 was higher when piglets were closer to the human and  
352 this effect was stronger for untamed piglets than tamed ones (away – close, H : z.ratio = -5.54  $p$   
353 < 0.001, H+ : z.ratio = -3.56  $p = 0.002$ , figure 3C), meaning the grunts were higher pitched when  
354 produced closer to the human. This effect did not remain true after the conditioning as no evidence of  
355 any difference between treatments and location was found ( $|z.ratio| < 2.4$   $p > 0.09$ , ). For AcPC3,  
356 post hoc tests did not reach significant levels ( $|z.ratio| < 2.3$   $p > 0.09$  for any comparison) .

357 **Emergence of positive perception of human (effect of additional positive contacts sessions**  
358 **along the conditioning)**

359 **The conditioning process increases behavioural proximity**

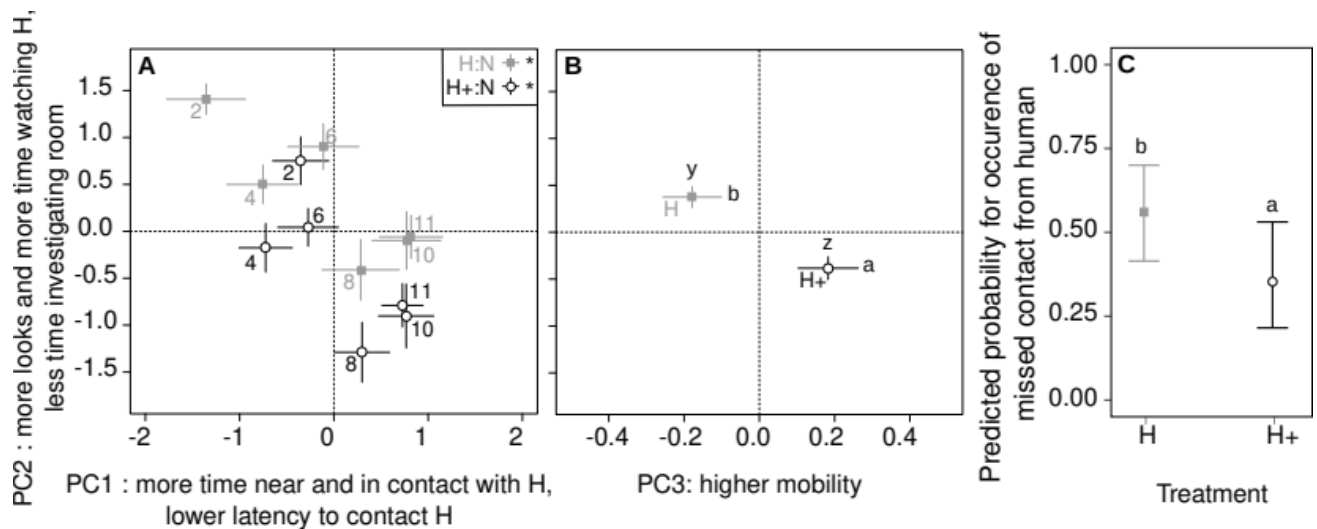


Figure 5: Effect of trial number over the conditioning on spatial behaviour and proximity to human during the 2min sessions of additional positive contacts during reunions of the conditioning. A and B: Mean  $\pm$  SE per group. A: Single effect of trial number on behavioural PC1 and PC2 according to treatment (H: grey, H+: black). B: Single effect of treatment on behavioural PC3 and PC2. C and D: predicted probability of occurrence of at least one failed contact from the human, mean estimates  $\pm$  95% confidence interval from the generalized mixed effect model. C: single effect of treatment. Stars in the legend box represent significant effect of the trial number (A), different letters represent significantly different groups (B and C). Full statistical report is available as supplementary material (tables S1 et S2 for statistical tests, table S3 for model estimates).

361 No evidence of any effect of the interaction between the treatment (tamed piglets before the  
 362 conditioning H+ or not H) and the trial number was found for all behavioural scores (CondPC1,  
 363 CondPC2 and CondPC3, table 3). Independently from the treatment, the higher the trial number was  
 364 the higher CondPC1 was ( $\chi^2_1 = 59.3$ ,  $p < 0.001$ , slope estimate [95% confidence interval]: 0.20  
 365 [0.15 : 0.25]) and the lower CondPC2 was ( $\chi^2_1 = 48.6$ ,  $p < 0.001$ , slope estimate: -0.17 [-0.22 : -  
 366 0.12]). According to the loadings, over the conditioning, piglets decreased the latency to contact the  
 367 human, made more contacts, spent more time in the proximal zone and in contact with the human  
 368 (condPC1), decreased the number of looks to the human, spent less watching the human and more  
 369 time investigating the room (CondPC2) (figure 4A). Independently from the trial number, tamed  
 370 piglets had a lower CondPC2 and a higher CondPC3 than the ones from the H group ( $\chi^2_1 = 12.8$ ,  $p <$   
 371  $0.001$  and  $\chi^2_1 = 7.0$ ,  $p = 0.008$  respectively), meaning that tamed piglets expressed a fewer number of  
 372 looks to the human, spent less time watching them and more time investigating the room (CondPC2)  
 373 and crossed more virtual zone during the test (CondPC3) (figure 4B). The probability of having at  
 374 least one missed contact by the human during a session was lower for tamed piglets than untamed  
 375 ones ( $\chi^2_1 = 9.57$ ,  $p = 0.002$ , figure 4C), with no interaction with the trial number ( $\chi^2_1 = 0.22$ ,  $p =$   
 376  $0.064$ ).

377

378 Grunt acoustic features depends on spatial proximity to human

379

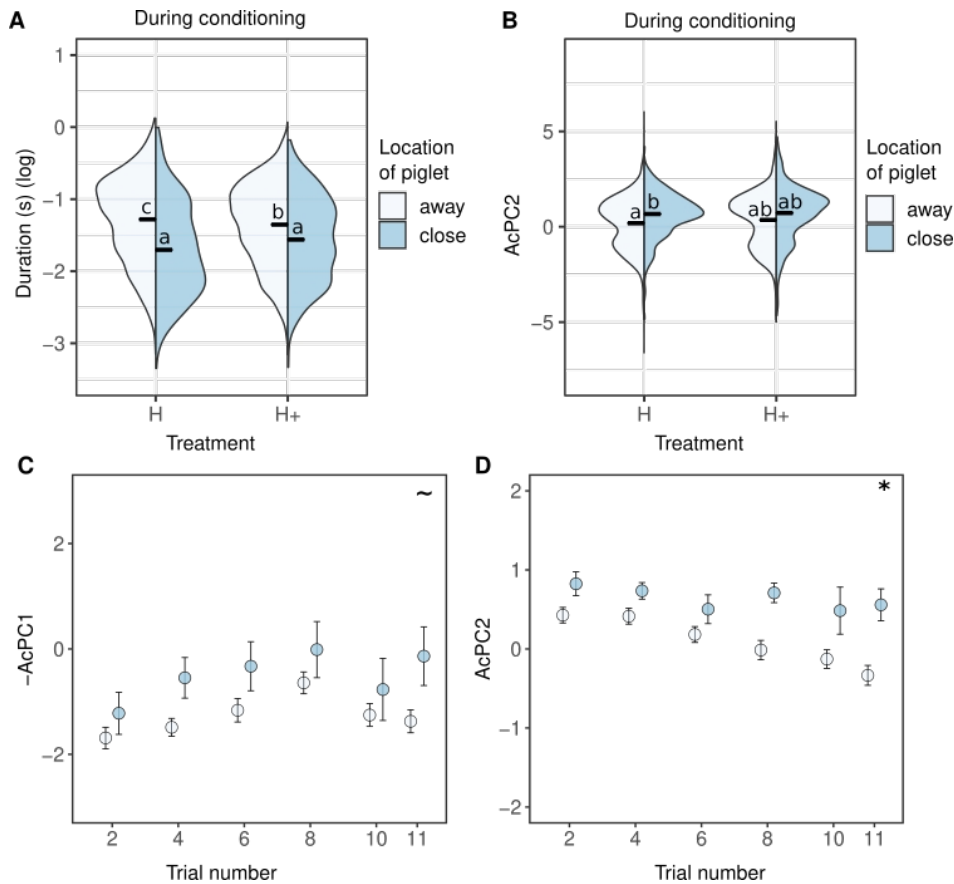


Figure 6: Evolution of acoustic parameters over the conditioning, depending treatment and location of piglets, during the 2min sessions of additional positive contact reunions.. A-B : Violin plots representing the median and the density of data distribution in the group. C-D : Mean +- SE per group, BA-B: Interacting effect of the treatment and the location of piglets (in proximal zone of the human: dark blue or away from the human: light blue ) on grunt duration (A) and AcPC2. C-D: Interacting effect of trial number and location of piglet on -AcPC1 (C) and AcPC2 (D). Different letters in A and B represent significantly different groups, "\*" in D represents significant difference between slopes and "~" in C a trend. . Full statistical report is available as supplementary material (tables S1-S3).

380 During the sessions of additional positive contacts of the conditioning, the three-way interaction  
 381 between the trial number, the treatment and the location was not significant for any of the acoustic  
 382 scores ( $\chi^2_1 < 0.18$   $p > 0.67$ ), allowing the analysis of the two way interactions of interest. The  
 383 interaction between treatment and the trial number was not significant for all acoustic scores ( $\chi^2_1 <$   
 384  $2.5$   $p > 0.11$ ), however grunt duration decreased over the conditioning sessions (trial  
 385 number:replicate interaction,  $\chi^2_1 \leq 5.3$   $p = 0.02$ , slope estimate  $-0.03[-0.04;-0.01]$  for the lower  
 386 slope, table S1 and S3). Independently from the trial number, grunt duration was lower when piglets  
 387 were located close to the human and this effect was stronger in untamed piglets than tamed piglets

388 (treatment:location interaction:  $\chi^2_1 = 15.8$   $p < 0.001$ , away vs. close, H piglets: z.ratio = 10.2  $p <$   
389 0.001, H+ piglets: z.ratio = 6.86  $p < 0.001$ , figure 5A). AcPC2 was higher when piglets were close to  
390 the human, but only in untamed piglets (treatment:location interaction,  $\chi^2_1 = 7.6$   $p = 0.005$ , pairwise  
391 comparisons away vs close, in H: z.ratio = -4.9  $p < 0.001$  and in H+: z.ratio = -2.0  $p = 0.21$ ), meaning  
392 that untamed piglets produced higher pitched grunts when closer to the human (figure 5B). The  
393 effect of the location on -AcPC1 and AcPC2 depended on the trial number (trial number : location  
394 interaction,  $\chi^2_1 = 3.97$   $p = 0.05$  and  $\chi^2_1 = 6.1$   $p = 0.01$  respectively): -AcPC1 and AcPC2 were higher  
395 when closer to the human with a greater extent later in the conditioning than earlier (slope  
396 comparison away – close, -AcPC1 : z.ratio = -1.80  $p = 0.07$ , AcPC2 : z.ratio = -2.34  $p = 0.02$ ).  
397 According to the loadings, this means that the frequency range, bandwidth and noisiness of grunts (-  
398 AcPC1) as well as the pitch (AcPC2) decreased over the conditioning when piglets were located  
399 away from the human but remained high when piglets were close (figure 5C and 5D).

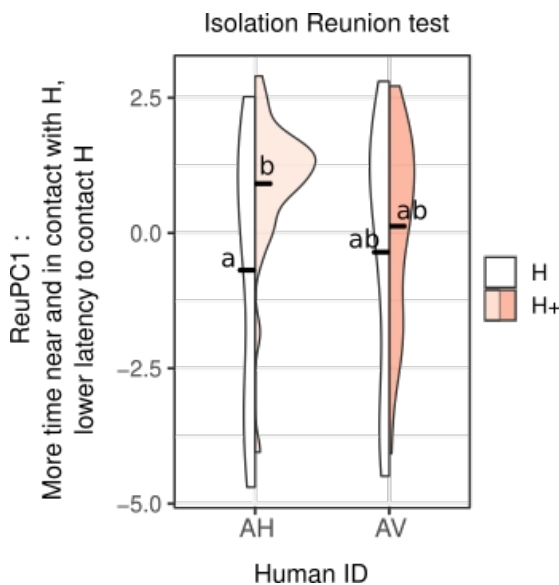


Figure 7: Effect of human identity on spatial behaviour and proximity during standard isolation reunion tests. Violin plots representing the median and the density of data distribution in the group. Different letters represent significantly different groups. Full statistical report is available as supplementary material (tables S1 and S2 for statistical tests, table S3 for model estimates).

401 Since half of the piglets had been assigned to one human experimenter and the other half to another  
402 one, this allowed the analysis of the effect of the identity of the human on behavioural and vocal  
403 scores. During the reunions of the Isolation/Reunion test, the behavioural proximity score (ReuPC1)  
404 was higher when piglets were tamed by the human ‘AH’ (treatment: human ID interaction,  $\chi^2_1 =$

405 6.01,  $p = 0.01$ , pairwise comparison H vs H+, AH:  $t$ -ratio = -4.77,  $p < 0.001$ , figure 6). When the  
 406 human ‘AV’ tamed the piglets, ReuPC1 scores exhibited intermediate values and not significantly  
 407 different score between treatments (AV, H vs H+:  $t$ -ratio = -1.33,  $p = 0.56$ ). This effect of the human  
 408 identity on behaviour was not found considering the reunions of the conditioning ( $\chi^2_1 < 1.32$ ,  $p >$   
 409 0.25 for all CondPCs, table S1).

410

411 *Table 5: Significant effects of human identity on vocal parameters (AcCP1 and AcPC2) during the reunion of the*  
 412 *Isolation/Reunion test and during the sessions of additional positive contacts of the conditioning. Only significant effect*  
 413 *are presented here but a full statistical report is available as supplementary material (tables S1 and S2 for statistical*  
 414 *tests, table S3 for model estimates). When single effects were interpretable, the Chi-squared statistic are reported, when*  
 415 *significant interactions were significant, post hoc pairwise comparisons were performed with Tukey corrected and are*  
 416 *thus reported. The estimates correspond either to the group estimate and comparisons of groups (categorical fixed effect)*  
 417 *or slope estimates and comparison of slopes (continuous fixed effect, ‘Trial number’).*

Acoustic parameter	Fixed effect	Levels	Estimate	Lower.95%CI	Upper.95%CI	Statistic	P-value
<b>Reunion of the Isolation/Reunion test</b>							
AcPC2	Human ID	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>							
-AcPC1	Human ID	AH	0.091	0.037	0.144	$\chi^2_1 = 4.69$	P = 0.03
		AV	0.076	0.021	0.132		
AcPC2	Human ID*In	AH – away	0.317	0.110	0.524	Z-ratio = -1.23	P = 0.60
		AH – close	0.402	0.161	0.643		
	prox. zone	AV – away	0.027	-0.182	0.236	Z-ratio = -5.77	P < 0.001
		AV – close	0.462	0.212	0.712		
AcPC3	Human ID * 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		

418

419 Considering the acoustic scores, no effect of human identity was found on AcPC1 during the  
 420 Isolation/Reunion tests but -AcPC1 was higher when the human ‘AH’ was in the room during the



421 reunion periods of the conditioning (table 5), meaning the frequency range and the bandwidth of the  
422 grunt were higher when the human ‘AH’ interacted with the piglet compared to the human ‘AV’.  
423 AcPC2 was higher when the human ‘AH’ was in the room during the Isolation/Reunion tests (table  
424 5), meaning that the pitch of grunts was higher and this effect was also found during the sessions of  
425 additional positive contacts of the conditioning in interaction with the location of the piglet ( $\chi^2_1 =$   
426 11.9,  $p = 0.001$ ): although, AcPC2 increased when piglets were located close to the human, this  
427 increase was significant only for the human ‘AV’ and not for ‘AH’ (table 5). AcPC3 was not different  
428 between humans during the reunions of the Isolation/Reunion tests but, over the conditioning,  
429 AcPC3 changed differently when piglets were tamed by the human ‘AH’ or ‘AV’, as showed by the  
430 significant interaction between trial number and human identity ( $\chi^2_1 = 8.0$ ,  $p = 0.005$ ): the skewness  
431 and kurtosis of grunts decreased over the conditioning when ‘AH’ was interacting with the piglets,  
432 but not ‘AV’ (see slope estimates, table 5). No evidence of any effect of human identity was found  
433 on grunt duration neither during the Isolation/Reunion tests nor during the sessions of additional  
434 positive contacts of the conditioning (table S1).

## 435 **Discussion**

### 436 ***Behavioural evidence of a rapid establishment of interest and proximity toward a human*** 437 ***providing additional positive contacts***

438 The standard reunion test with the human before the conditioning showed that the taming treatment  
439 succeeded in creating two different levels of human-piglet relationship (H and H+), as tamed piglets  
440 expressed a higher attractiveness toward the human (ReuPC1) than untamed piglets, parameters  
441 considered as indicators of a positive HAR (Rault et al. 2020). This test also showed that the  
442 conditioning modified the behaviour of untamed piglets so that they finally expressed a similar  
443 attractiveness toward the human as tamed piglets, after the conditioning. So it seems that the  
444 conditioning process allowed untamed piglets to compensate the lack of taming before the  
445 conditioning and develop a positive perception of the human. In addition, tamed piglets expressed  
446 more exploratory behaviours than untamed piglets after the conditioning (ReuPC3), which may be  
447 interpreted as natural foraging and disinterest from human contact, which may be a sign of positive  
448 welfare (Weerd and Day 2009). However, this could be interpreted also in terms of attachment to the  
449 human. Indeed, attachment to a human may facilitates exploration of novel environments or objects,  
450 as shown in dogs (Palmer and Custance 2008). Attachment has already been hypothesised in the  
451 lambs-human relationship (Tallet, Veissier, and Boivin 2009).

452 The analysis of piglets' behavior every second sessions of the conditioning showed that, over time  
453 and for both treatments (H and H+), piglets expressed less fear (CondPC2), a higher attraction  
454 toward the human (CondPC1) and avoided less the human when the later attempted to interact with  
455 them. As a conclusion, two- minute daily sessions of additional positive contacts did change  
456 positively the perception of the human for the piglets, and thus their willingness to interact with  
457 them. Since no evidence of any interaction between time and treatment was found, no conclusion of  
458 differential developmental trajectories between treatments can be drawn, but a parallel development  
459 of the human-piglet relationship in both groups, when considering the conditioning sessions (and not  
460 the standard reunion tests). Over the conditioning sessions and similarly to the observations during  
461 the standard reunion test, tamed piglets expressed higher mobility and room investigation  
462 behaviours. This may allow us to generalize our hypothesis on attachment described above : taming  
463 may provide an environment secure enough for the piglets to explore their environment in the  
464 presence of the human. Overall, the behavioural monitoring showed that two minute sessions of  
465 positive additional contacts per day are sufficient to increase proximity to a human to similar levels  
466 as when piglets were previously familiarised for 2 weeks, even when piglets experienced social  
467 isolation, but it did not allow the piglets to express natural exploratory behaviours as the previously  
468 tamed piglets.

#### 469 **Links between vocal expression and positive HAR**

470 In this study, piglets were subjected to two type of interactions with the human : during the standard  
471 reunion test, no movements nor speech was produced by the human, during the sessions of additional  
472 positive contacts of the conditioning, the human provided contact and produced speech toward the  
473 piglet. These types of interactions had different effects on vocal expression, which allow us to  
474 evaluate the origin and functions of the vocal flexibility expressed in grunt structure, first studying the  
475 standard reunion test first and the sessions of additional positive contacts after.

#### 476 **Human mere presence affects vocal expression according to previous experience**

477 Social isolation was associated to longer and lower pitched grunts with a down shifted frequency  
478 spectrum. A reunion with a static human changed grunt structure to shorter, higher pitched with an  
479 up shifted frequency spectrum and this was observed independently from the treatment (H or H+)  
480 and the conditioning time (before or after). In terms of emotional indicators, similar changes in  
481 acoustic features of grunts were already found in studies focusing on vocal markers of valence in pig  
482 (Briefer et al. 2019; 2022; Avelyne S. Villain et al. 2020a; Friel et al. 2019)and are also in line with  
483 previous results in similar contexts in relation to the human presence (Avelyne S. Villain et al.

484 2020b). These results, combined with the behavioural results, validate the vocal parameters encoding  
485 of positive emotions in the context of a reunion with a human.

486 In addition, and surprisingly, tamed piglets produced shorter grunts than untamed piglets whatever  
487 the context of observation (with or without human presence). This was previously shown in another  
488 context (anticipation of (pseudo)social events independently from the type of partner) in the same  
489 groups of pigs (Villain et al. 2020). This may show that taming modulates general vocal production  
490 in pigs on a long term.

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

511 The results on vocal expression during the standard reunion test show that even in a context in which  
512 the human does not provide positive contacts, the experience the piglets previously had with her  
513 affected their vocal expression when close to her and on the long term. On the one hand, we know a  
514 positive HAR establishes through successive positive experiences (Rault et al. 2020) (Rault et al.  
515 2020) and, on the other hand, HAR may have long term effects on behavioural expressions, as

516 suggested by Brajon et al. (2015) using cognitive bias tests. We can thus hypothesize this may also  
517 be reflected in the way piglets vocally express. In that case, we may have evidence of expression of  
518 another category of affect, moods, and not only emotional expression. Indeed, as suggested by  
519 Schnall (2010), although emotions are short term affects triggered by an external stimulus, moods, on  
520 the other hand may be experienced on a longer term and may not be attributable to a specific  
521 stimulus. Although emotions and moods do not express on the same time scale they may interact  
522 with one another and more studies need to be carry out to disentangled their effects on vocal  
523 expression.

524 However, this test led to two potential hypotheses to explain why effect of human proximity on grunt  
525 acoustic parameters attenuated as the familiarity to the human increased (decreasing in reactivity  
526 along with increasing in foraging natural behaviours or violation of piglets expectations with a static  
527 human, inhibiting vocal reactions to the proximity). The interpretation of the second type of human-  
528 piglet interactions may allow to address these hypotheses.

#### 529 Providing rewarding additional positive contacts changes the structure of grunts

530 During the sessions of additional positive contacts of the conditioning, independently from the trial  
531 number, the duration of grunts was lower when the piglets were located close to the human and  
532 similarly short in both experimental treatments, but when away from the human, untamed piglets had  
533 longer grunts than tamed piglets. In addition, although the pitch (AcPC2) and the frequency  
534 distribution (-AcPC1) increased over the conditioning, it remained higher when grunts were  
535 produced closer to the human and especially in untamed piglets (AcPC2). These results are in line  
536 with the behavioural results showing an increase in proximity to the human over the conditioning  
537 (CondPC1). During this type of interaction, and contrary to the standard reunion test, changes in  
538 acoustic features of grunts when close to the human were consistent over the conditioning: piglets  
539 remained reactive to the proximity to the human over time. This result may not be in line with the  
540 first hypothesis : in the case of a human interacting with a piglets, the effect of the proximity does not  
541 seem to attenuate over time. Hence, the behaviour of the human during a session impacts the way a  
542 piglet vocalises. In that case, we may raise two more hypotheses to explain this vocal behaviour :  
543 either it is linked to the emotional state or it is linked to a specific human-animal communication, the  
544 two explanations may not be exclusive. The first possible explanation may be linked to the emotional  
545 state experienced by the piglets when approaching a human providing additional positive contact. As  
546 a reminder, in the context of the session, the piglet can chose to approach and stay close to the  
547 human, which will provide positive contacts systematically. So the piglet may anticipate to receive

548 positive contact and systematically being rewarded. When close to the human, observed changes in  
549 frequency distribution of grunts (increased pitched and up shifted frequency spectrum) are known to  
550 be markers of arousal (in multiple mamalian species (Briefer 2012; 2020) and pigs (Linhart et al.  
551 2015)). As a consequence, this may show that piglets enter a state of higher arousal when being close  
552 to a carying human. To go furhter, we show that these spectral changes were also associated with  
553 shorter grunts. Although the duration of grunts is associated to the valence of a situation, it is not  
554 clear whether the duration could also be an indicator of the arousal, if our hypothesis is true then it  
555 would be the first demonstration that shorter grunts are also indicators of higher arousal positive state  
556 in pigs. Last, we can raise the question whether changes in grunt structure may also be associated to  
557 a specific human-piglet communication. In other domestic species, owner directed vocalisations has  
558 been shown (in cats, reviewed in (Turner 2017); in dogs (Gaunet, Savalli, and Legou 2022)). In  
559 addition, paraleles studies have found similar socio-communicative behaviours toward a human in  
560 socialized pigs and dogs (Gerencsér et al. 2019). Hens, we may profit from testing the existence of  
561 human directed vocalisations in pigs, as consequences of their socio communicative abilities.

### 562 **Effect of human identity on piglets' perception : perspectives on HAR**

563 We found that the identity of the human had effects on behavioural and vocal parameters. Piglets  
564 tamed by the human 'AH' had higher values of behavioural proximity (ReuPC1) than piglets tamed  
565 by the human 'AV' during reunion test after a period of isolation. This effect was not found during  
566 conditioning sessions. Additionally, when the human 'AH' was in the room, piglets produced grunts  
567 with a more upshifted frequency spectrum and a higher pitch than when the human 'AV' was in the  
568 room, leading to the conclusion that 'AH' was more entitled to trigger higher positive states than  
569 'AV'. Interestingly, the effect of spatial proximity depending the identity of the human on piglets  
570 grunts was found during the sessions of additional positive contacts but not when the human was  
571 static during the standard reunion test. Hence, it is possible that the way one human interact  
572 (behavioural and vocally) with a piglet may be more or less effective at triggering positive emotions  
573 and thus modifications of grunt structure. Several evidence exist in the literature that pigs  
574 discriminate humans visual and auditory cues (Brajon et al. 2015c; Bensoussan et al. 2019). Pigs  
575 may also show behavioural changes hearing human voice (Bensoussan et al. 2020). We may question  
576 the efficiency of different human features to generate a positive HAR. In our study, both humans that  
577 interacted with the piglets wear exactly the same clothes and standardized their tactile interactions  
578 toward the piglets before starting the study, and agree on the rhythm and types of sounds (the words  
579 used) to use, to minimise generating variability although no systematic controls of the human

580 behaviour or spectral feature of voices were performed here. It thus remains unclear whether  
581 experimenters interacted differently or if they were initially perceived differently by piglets. Studies of  
582 human features that are most likely to generate a positive HAR may be of interest regarding animal  
583 welfare. In addition, studying human-pig relationship in a more systematic way, as in other domestic  
584 species with example of play behaviour in dogs (Horowitz and Hecht 2016) or the acoustic example  
585 of the pet directed speech (Lansade et al. 2021; Jeannin et al. 2017), may shed light on the evolution  
586 and converging strategies of interspecific relationships. However, the influence of human identity did  
587 not modify the general outcomes of our study, but only decrease some effects, suggesting that this  
588 variability does not modify the main results, but should be considered in future studies.

589

590 To conclude, we showed that degrees of familiarity toward a human could be reflected in the way  
591 piglets vocalise in their presence, and out of it. We also showed that the spatial proximity toward a  
592 human providing additional care could change the acoustic structure of piglet grunts. These changes  
593 are likely to be linked to positive and more intense emotional states that when piglets are further  
594 away from the human. However, it is still unclear whether the changes in grunt structure could also  
595 be linked to human-animal communication and more studies are needed to determine it. We did also  
596 show that the identity of the human may be of important, generating specific vocal changes during  
597 additional positive contacts that were not associated to changes in behaviour. More systematic  
598 studies of human behaviour along with pig behaviour during the human-animal interactions would be  
599 needed to have a better understanding of the evolution of HAR, especially interactive interspecific  
600 communication as well as providing new procedures to promote positive welfare. We suggest that  
601 the use of vocalisations to assess quality of human-pig relationship could help to better monitor the  
602 parameters involved in the establishment and maintenance of a positive HAR.

## 603 **Authors contributions**

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

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613

## 614 **Data availability**

615 The datasets used and/or analysed during the current study are available at (Villain et al. 2022).

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