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

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Abstract:

Studying human-animal interactions in domestic species and how they affect the establishment of a positive Human-Animal Relationship (HAR) may help us improve animal welfare and better understand the evolution of interspecific interactions associated with the domestication process. Understanding and describing the quality of an HAR requires information on several aspects of the animal biology and emotional states (social, spatial and postural behaviours, physiological and cognitive states). Growing evidence shows that acoustic features of animal vocalisations may be indicators of emotional states. Here, we tested the hypothesis that the quality of vocal expression may indicate the quality of HAR. At weaning, 30 piglets were positively handled by an experimenter who talked to and physically interacted with them three times a day, while 30 other piglets only received the contact necessary for proper husbandry. After two weeks, we recorded the behaviours and vocalisations produced in the presence of the static experimenter for five minutes. We repeated this test two weeks later, after a conditioning period during which human presence with

additional positive contact was used as a reward for all piglets. We hypothesized this conditioning period would lead to a positive human-piglet relationship for all piglets. As expected, piglets that were positively handled at weaning expressed a higher attraction toward the experimenter, and, after the conditioning, piglets that were not positively handled at weaning expressed a similar level of attraction than the positively handled ones. Piglets positively handled at weaning generally produced shorter grunts than the other ones. However the latter expressed more flexibility in call structure when vocalising close to a human, with a decrease of grunt duration and an increase in pitch, frequency range and noisiness in their grunt. This differential effect of proximity between groups of piglets was attenuated after the conditioning during a standard reunion with a static human but remained over time when the human was providing additional positive contacts. Results suggest that first, changes in vocal structure are consistent with indicators of positive states in the presence of a human. Second, increasing familiarity and proximity between a human and a pig may induce changes in the acoustic structure of its grunts. Third, a human providing additional positive contacts triggers more changes in vocalisation structure than by their presence only. We show that vocalisation structure may allow us to assess the quality of human-pig relationship.

Introduction

The process of domestication was conducted to shape physiology and morphology of domestic animal species, but also their behaviour. It notably has shaped interspecific interactions between human and non-human animals, by improving animals' capacity to use human signals to adapt their behaviour both decreasing fearfulness toward humans and increasing attention toward humans (Mignon-Grasteau et al., 2005). In farms, the relationship that domestic animals form with humans is important for animal welfare. Therefore, studying human-animal interactions and their consequences to understand the mechanisms of emergence and maintenance of a positive human-animal relationship (HAR) directly applies to welfare (Rault et al., 2020). Animal welfare consists of three major aspects: the ability of an animal to control its mental and physiological stability (Broom, 2011), the decrease of experiencing negatively perceived contexts and the increase in experiencing positively perceived contexts and species-specific behaviors (Peterson et al., 1995; Weerd & Day, 2009). A positive HAR is thought to be established through repeated positive interactions between the human and the non-human animal. Some of the mechanisms involved in this process are:

accumulation of positive experiences through positive associative learning, modifications of cognitive biases, shaping expectations from the non-human animal toward the human. A positive HAR can be appreciated through behavioural and physiological measures, for example by assessing the expression of positive emotions [reviewed in (Rault et al. 2020)]. Several behavioural measures may help to define a positive HAR such as: short latency to approach and spatial proximity (Boivin et al., 2000; Schmied et al., 2008), body postures (Villain et al., 2020b) or play behaviour (Jerolmack, 2009). Contacts from a human such as stroking, may induce changes in body postures and exposition of body areas by the animal to the human, supposedly vulnerable [central neck area in cattle (Schmied et al. 2008), abdominal area in pigs (Rault et al., 2019)]. Such grooming solicitation may be markers of engagement, trust and motivation to interact with the human. In most cases, these behaviours are similar to those shown during intraspecific socio positive interactions, although there are some species specific behaviours [e.g., dog vs.. wolf (Gácsi et al., 2005)]. Vocal behaviour may also help defining the quality of an HAR. First, vocalisations are known to carry markers of the emotional states in several bird and mammal species (Briefer, 2012, 2020). Markers of emotional valence (positive versus negative) has been studied in domestic farm animals [reviewed in Laurijs et al. (2021)]. Second, some vocalisations have been associated with positive interactions with humans, for example the cat – human communication : purring is thought to be derived from mother pup communication during nursing and is observed associated with care solicitation from humans; meowing, which is not observed during intra specific interactions is thought to emerge from associative learning during cat – human interactions (Brown & Bradshaw, 2014). This shows that HAR may elicit specific vocalisations from the non human animal toward the human.

In pigs, diversified evidence attest the possibility of a positive HAR. Animals may be handled by humans providing regular additional positive contacts, leading to the expression of a positive perception of humans, with evidence from behavioural and physiological studies. Cognitive bias tests showed a positive judgment bias in piglets that had received gentle contacts with humans (Brajon et al., 2015b). Pigs may recognise a human providing positive contacts compared to an unfamiliar one and adapt their behaviour accordingly (Brajon et al., 2015c). Pigs may be sensitive to human voice and respond accordingly (Bensoussan et al., 2019, 2020). Pigs vocalisations are diverse and linked to their emotional states, attested by the use of positive or negative call types (Briefer et al., 2019, 2022; Tallet et al., 2013). In addition, even within a call type, spectro-temporal changes are closely related to the valence of a situation or the arousal a situation may trigger for the animal. For example, grunts, that are among of the most used vocal signals and various situations is now known to be a flexible call: shorter grunts have been associated with positive situations (Briefer et al., 2019, 2022;

Friel et al., 2019), as well as higher formants (which are frequency peaks containing more energy than others) and a lower fundamental frequency during positive situations (Briefer et al., 2019, 2022). Grunt structure may also change according to the arousal of a situation, with a higher frequency range and a higher bandwidth when the arousal increases (Linhart et al., 2015). In order to determine if vocalisations may be used as non invasive indicators of the quality of human-pig relationship by themselves, we tested whether they could encode the quality of the human-pig relationship, through the vocal expression of emotional state. Because they are used in contexts of different valence and arousal and in most pigs, we studied the spectro-temporal structure of grunts. We predicted that if grunts carry information on the quality of the human-pig relationship, then 1. A period of positive handling given by a human should modulate vocal quality of piglets when in presence of the human, leading to grunts exhibiting markers of positive states (shorter grunts), 2. spatial proximity toward the human should influence the spectro-temporal structure of grunts (higher pitched grunts as the arousal increases).

Methods

Ethical note

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

Subjects and housing conditions

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

One piglet had to be excluded from our sample size to receive care/medication due to health issues independent from the experiment. From weaning at 28 days of age, piglets from the same litter and having similar weight (<1 kg difference) were housed by three in a 1.2 x 1.3m pen on plastic

duckboard. Wooden panels were used to visually isolate pens. One metal chain per pen was used for enrichment. Food and water were available *ad libitum*. Artificial lights were turned on from 8:00 to 17:00 and temperature was maintained between 26 and 27 ºC. The experiment was carried out in two replicates and two identical rearing rooms were used (5 pens per room per replicate).

Treatment: positive handling et weaning

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

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

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

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

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

The conditioning took place between day 42 and 62 of age and lasted twelve days, with two trials per day and at least three hours between trials on the same day. Piglets were habituated to the test room for 10 minutes, by pen, two days before the start of the conditioning. All piglets (H and H+) were

subjected to the same conditioning. The experimental design of the conditioning is already published in an article dedicated to the study of anticipatory behaviour (Villain et al., 2020).

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

The same sessions occurred in both treatment groups (H and H+). It was thus excepted that, at the end of the conditioning, all piglets would be familiar with the human, but with a different degree in H+ and H- piglets, due to a different time of exposure (H+: period of positive handling at weaning + conditioning, H: conditioning only). Sessions of reunions with social partners were not studied and only served as reward during the conditioning in a previous analysis of vocal expression of positive anticipation (Villain et al., 2020). Indeed, first the two (pseudo) social contexts would have been difficult to compare (reunion between three piglets vs. reunion between one piglet and one human). Second, regarding the vocal behaviour, the caller among the group of three piglets would not have been identified reliably, making it difficult to study within individual vocal flexibility.

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

Standard Isolation/Reunion Tests

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

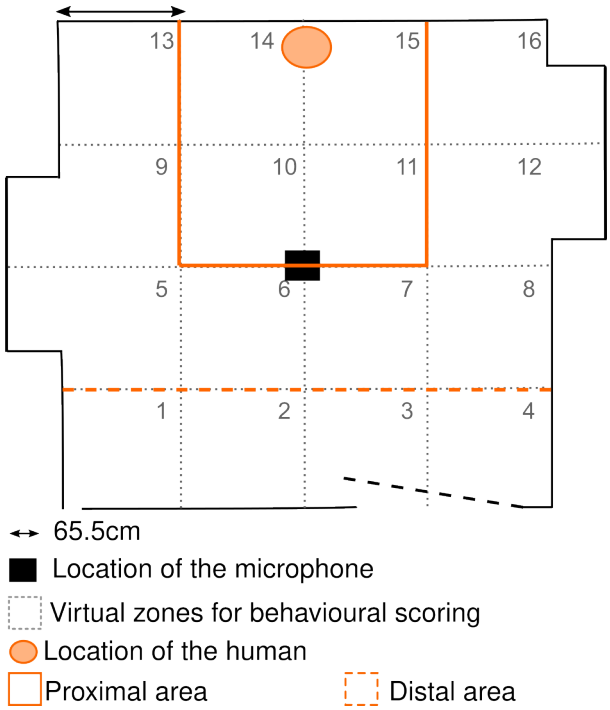


Figure 1: Design of the room used during the Isolation/Reunion tests and the additional positive contacts sessions of the conditioning. The room was split into 16 virtual zones. A proximal area (zones 10, 11, 14, 15) and a distal area (zones 1, 2, 3, 4) were defined, suing the location of the human as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

Behavioural monitoring and analyses

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

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

Table 1: Ethogram.

Behaviour	**Description**
Nb zones crossed [1,2]	The number of times the piglet crossed a virtual zone
Nb approaches H [1]	Number of times the piglets entered the proximal area
Time watching H [1,2]	The amount of time the piglet spent watching the human
Latency to contact H [1,2]	The latency to the first contact of the human by the piglet

Nb looks toward H [1,2]	The number of times the piglet turned its head toward the human
Nb looks other than H [1]	The number of times the piglet looked at other parts of the room than the human or the floor (walls, doors)
Time watching room [1]	The number of times the piglet watched other parts of the room than the human or the floor (walls, doors)
Time in proximal area [1,2]	The amount of time the piglet spent in the proximal area
Time in distal area [1,2]	The amount of time the piglet spent in the distal area
Time in contact H [1,2]	The amount of time the piglet investigated the human
Time investigating floor [1,2]	The amount of time the piglet investigated the floor
Nb contacts H [2]	Number of times the piglet was in contact with the human (initiated by the piglet or the human)

[1]: Scored during reunions of Isolation/Reunion tests. [2]: Scored during reunions of conditioning sessions

Acoustic monitoring and analyses

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

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

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

Statistical analyses

Behavioural and vocal response scores

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

Table 2: Percentage of explained variance and relative loadings of parameters on PCs, following the Principal Component Analysis computed on the behaviours scored during the reunion of the Isolation/Reunion test. The first three PCs, having an eigenvalue above 1, constituted three behavioural scores: ReuPC1, ReuPC2, ReuPC3. Parameters that explain the most each PC are bolded (|loading|>0.4).

	ReuPC1	**ReuPC2**	**ReuPC3**
Cumulative variance explained %	38.3	60.8	74
Nb of zones crossed	24.177	**-55.843**	-0.435
Nb approaches H	**47.748**	-30.163	0.578
Time watching H	**-52.914**	-7.422	25.585
Latency to contact H	**-64.232**	-0.464	1.688
Nb looks toward H	-7.787	**-43.721**	31.633
Time watching room	-32.048	-13.581	-6.238
Nb looks other than H	3.524	**-72.408**	-2.027
Time in proximal area	**69.96**	-0.156	9.584
Time in distal area	**-46.416**	-12.437	-1.215
Time in contact H	**61.041**	3.586	24.183
Time spent investigating floor	11.868	-7.503	**-42.265**

Table 3: Percentage of explained variance and relative loadings of parameters on PCs, following the Principal Component Analysis computed on the behaviours scored during the sessions of additional positive contacts of the conditioning. The first three PCs, having an eigenvalue above 1 constituted three behavioural scores: CondPC1, CondPC2, CondPC3. Parameters that explain the most each PC are bolded (|loading|>0.4).

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

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

	VocPC1	VocPC2	VocPC3
Cumulative variance explained %	59.769	76.807	87.712
Mean Dominant Frequency[1]	-13.558	**53.557**	2.220
Min frequency peak[1] (log)	-0.349	**58.758**	24.236
Max frequency peak[1]	**-43.023**	8.760	-9.537
Mode[2] (log)	-0.522	**66.248**	19.268
Mean[2] (log)	**-95.092**	-2.295	2.028
Q50[2] (log)	**-85.278**	0.280	-0.093
Q25[2] (log)	**-52.360**	19.327	0.985
Q75[2] (sqrt)	**-88.925**	-4.645	2.309
Centroid[2] (log)	**-95.092**	-2.295	2.028
Sd[2]	**-64.484**	-11.303	7.680
IQR[2]	**-87.981**	-5.851	2.640
Sfm[3] (sqrt)	**-94.344**	-3.189	0.962
Sh[3] (sqrt)	**-96.087**	-0.785	-0.175
H[3]	**-88.205**	-1.059	-1.063
Skewness[4]	28.032	-18.010	**48.652**
Kurtosis[4]	22.973	-16.241	**50.615**

[1]: parameters related to the pitch of the vocalisation; [2]: parameters related to the frequency distribution descriptors; [3]: parameters related to the noise component of the vocalisation; [4]: parameters related to the shape of the frequency distribution

Statistical models

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

of additional positive contacts of the conditioning (but always the same human was attributed to a given piglet). The piglet was used as random factor to take into account the within-subject design.

Isolation/Reunion tests

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

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

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

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

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

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

Conditioning trials

The aim was to study the evolution of human-piglet relationship over the conditioning [the variable 'Trial number', used as a continuous variable], depending on the previous experience piglets had with the human [either already familiar (H+ group) or unfamiliar (H group) at the beginning of the conditioning]. Trial number (Trial) was also used as a random slope to take into account individual trajectories (Schielzeth and Forstmeier 2009). The following model was built to test the behavioural

response scores (lmer) and the occurrence of missed contact initiated by the human during a session (presence/absence, binomial model, glmer):

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

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

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

Model validation and statistical tests

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

Results

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

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

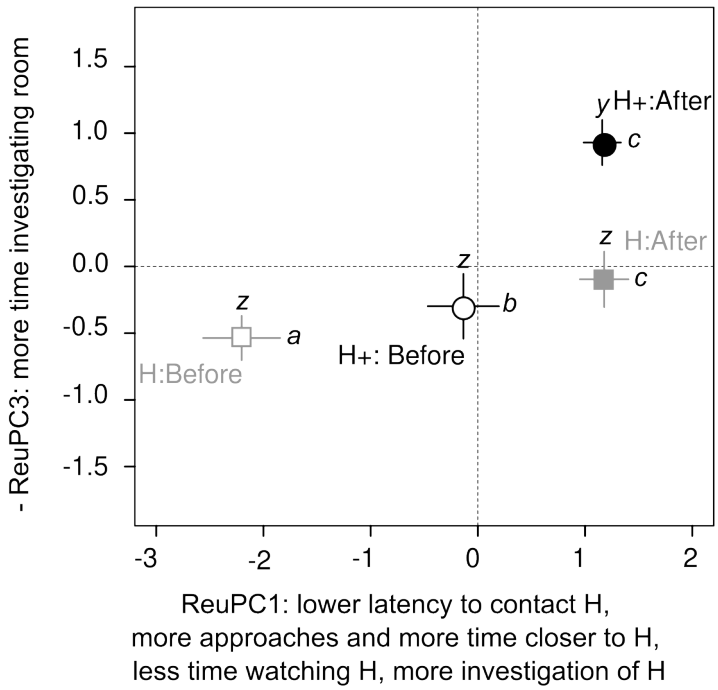


Figure 2: Effect of conditioning and treatment on spatial behaviour and proximity toward the human during the reunion of the Isolation/Reunion test. Mean ± SE per group is indicated, different letters indicates significantly different groups. Significant interaction between treatment (H : grey squares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

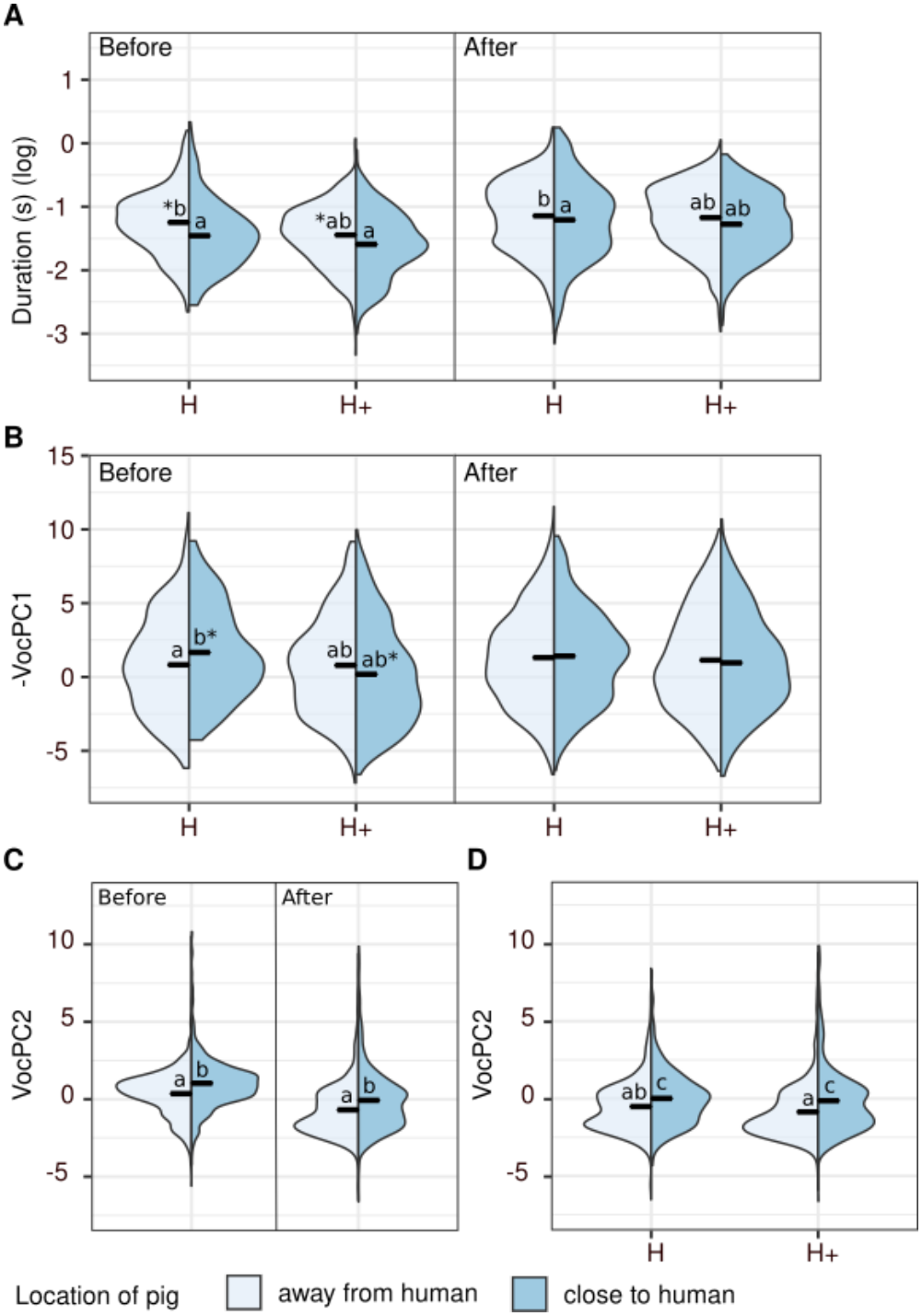
The interaction between the treatment (positively handled piglets before the conditioning (H+) or not (H)) and the conditioning time (before or after the conditioning) was significant for both ReuPC1 and ReuPC3 ($\chi^2_1 = 28.0$, $p < 0.001$, and $\chi^2_1 = 3.7$, $p = 0.05$ respectively, figure 2)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

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

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

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

392 *The conditioning process attenuates the effect of proximity on grunts vocal features in*
393 *non handled piglets*
394



395 *Figure 3: Acoustic structure of grunt during the reunions with a silent and static human (Isolation/Reunion test). Effect*
396 *of conditioning (before or after), treatment (H or H+), and location of the piglet relatively to the human (close: dark blue*
397 *or away from them: light blue). Violin plots representing the median and the density of data distribution in the*
398 *considered groups. (A, B) Results of post hoc tests following the significant three way interaction between the treatment,*
399 *the conditioning time and the location on grunt duration (A) and on the first vocal score (-VocPC1, B). (C,D) Results of*
400 *post hoc tests following the significant two way interactions between conditioning time and location (C) and between*
401 *treatment and location (D) on the second vocal score (VocPC2). When involved in interaction, the conditioning time was*
402 *fixed (as it was relevant to consider difference affected only by time). It thus allowed pairwise comparisons of interacting*

location and treatment (A, B) or levels of location (C). Letters represent significantly different groups (p < 0.05). When no letters are present, no significant difference between groups was found. Stars (*) between two groups represent a statistical trend (p< 0.10). Full statistical report is available as supplementary material (tables S1 S2 for statistical test and S3 for model estimates).

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

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

The conditioning process increases behavioural proximity

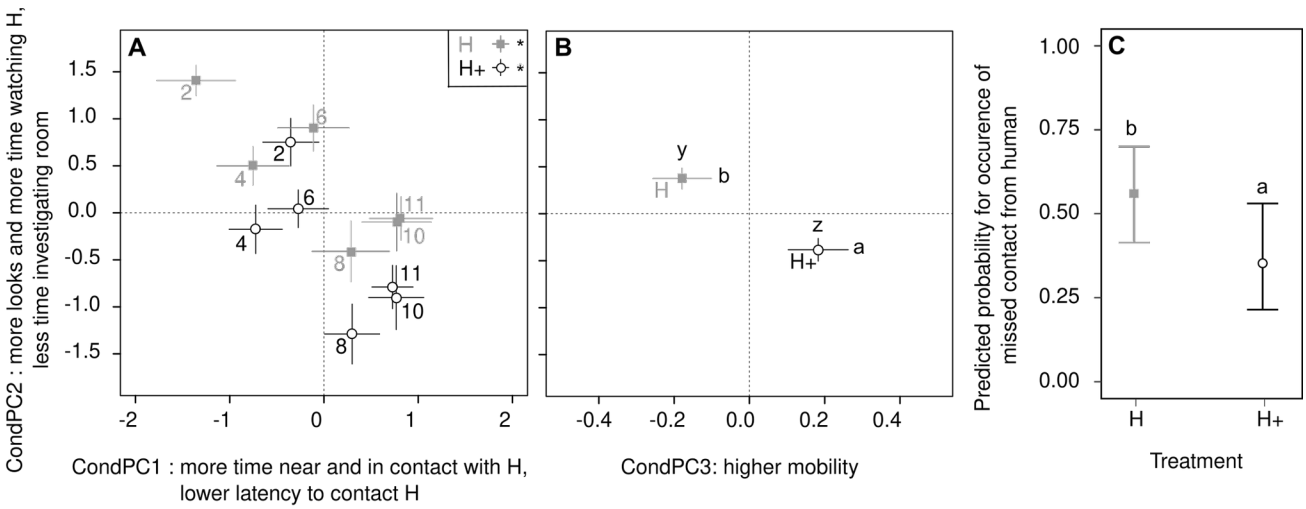


Figure 4: Effect of trial number over the conditioning on spatial behaviour and proximity to the human during the 2min sessions of additional positive contacts of the conditioning. (A, B) Mean ± SE per group. (A) Single effect of trial number on behavioural scores CondPC1 and condPC2 according to treatment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

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

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

Grunt acoustic features depends on spatial proximity to human

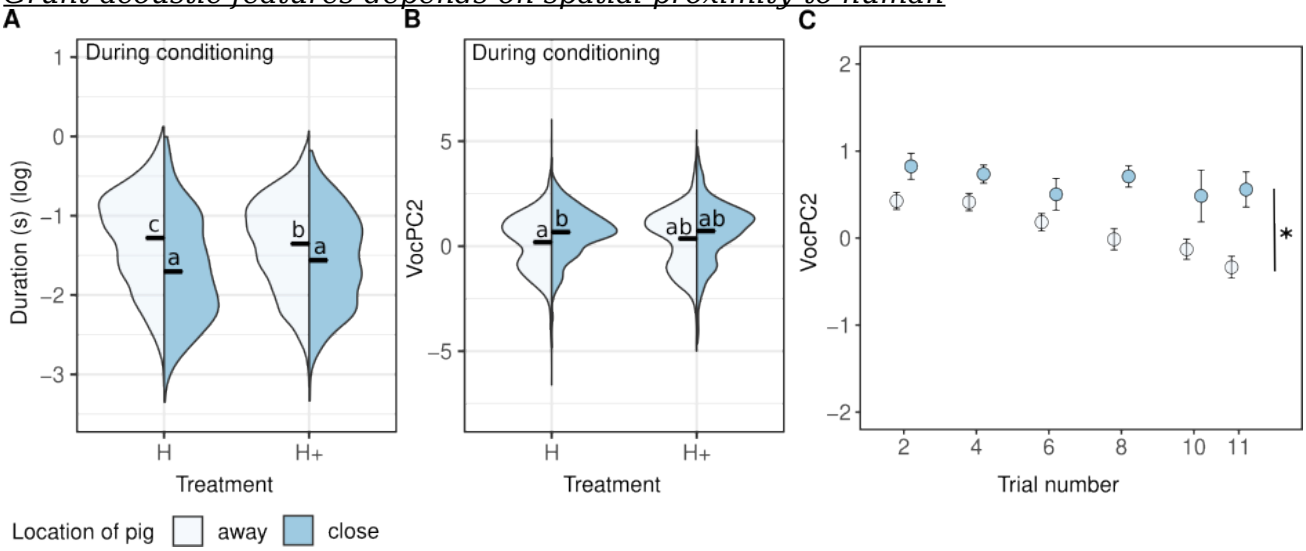


Figure 5: Evolution of vocal scores over the conditioning, during the 2min sessions of additional positive contacts. (A, B) Violin plots representing the median and the density of data distribution in the group. Interacting effect of location (in proximal area of the human '(close': dark blue) or elsewhere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

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

number : location interaction, $\chi^2_1 = 3.97$ p = 0.05 and $\chi^2_1 = 6.1$ p = 0.01 respectively): -VocPC1 and VocPC2 were higher when closer to the human with a greater extent later in the conditioning than earlier (slope comparison away – close, -VocPC1 : z.ratio = -1.80 p = 0.07, VocPC2 : z.ratio = -2.34 p = 0.02). According to the loadings, this means that the frequency range, bandwidth and noisiness of grunts (-VocPC1) as well as the pitch (VocPC2) decreased over the conditioning when piglets were located away from the human but remained high when piglets were close (figure 5C and 5D).

<u>Impact of human identity on piglets behaviour and grunt structure</u>

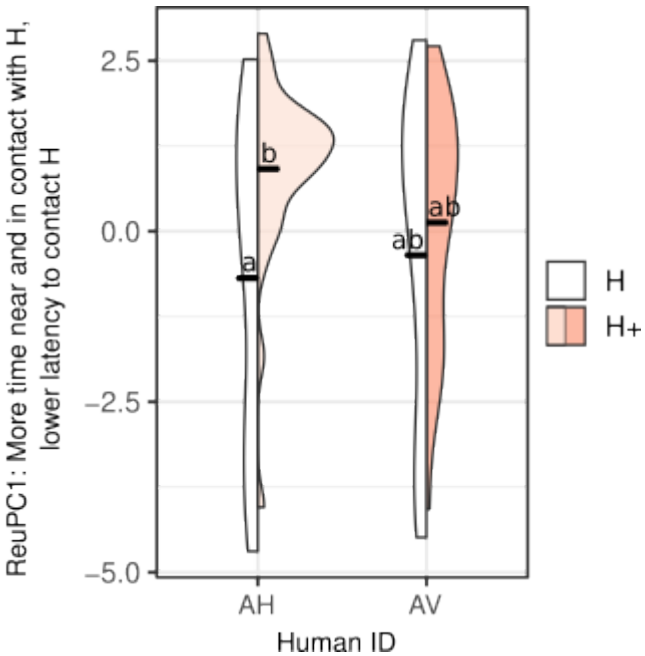


Figure 6: Effect of human identity on spatial behaviour and proximity during the reunion of the Isolation/Reunion test. Violin plots representing the median and the density of data distribution in the group. Different letters represent significantly different groups. Full statistical report is available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

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

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

intermediate values, treatment was not significant (AV, H vs. H+: t.ratio = -1.33, p = 0.56). These interacting effects of the human identity and treatment on behaviour were not found when considering the reunions of the conditioning ($\chi^2_1 < 1.32$, p > 0.25 for all CondPCs, table S1).

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

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

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

Considering the vocal scores, no effect of human identity was found on VocPC1 during the Isolation/Reunion tests but -VocPC1 was higher when the human 'AH' was in the room during the reunion periods of the conditioning (table 5), meaning the frequency range and the bandwidth of the

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

Discussion

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

The standard reunion test with the human before the conditioning showed that the treatment of positive handling at weaning succeeded in creating two different levels of human-piglet relationship (H and H+), as positively handled piglets expressed a higher attraction toward the human than non handled piglets (ReuPC1), parameters considered as indicators of a positive HAR (Rault et al., 2020). This test also showed that the conditioning modified the behaviour of non handled piglets so that they finally expressed a similar attraction toward the human as positively handled piglets, after the conditioning. These results are in line with the behavioural results of the sessions of additional positive contacts. The analysis of piglets' behaviour every second sessions of the conditioning showed that, although positively handled and non handled piglets started with different degree of proximity toward the human (trials 2 and 4, CondPC1), then, over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human (CondPC1) and avoided less the human when the latter attempted to interact with them. At the end of the conditioning, piglets from both groups had similar level of proximity toward the human (trials 8, 10, 11 CondPC1). So it seems that the conditioning process allowed non handled piglets to compensate the lack of positive handling before the conditioning and develop a similar proximity toward the human. Two minute daily sessions of additional positive contacts changed positively the perception of the human for the

552 piglets, and thus their willingness to interact with them. Since no evidence of any interaction
553 between time and treatment was found, no conclusion on differential developmental trajectories
554 between treatments can be drawn, but a parallel development of the human-piglet relationship in
555 both groups, when considering the proximity.

556 Beside behavioural proximity, piglets that were positively handled at weaning expressed more
557 exploratory behaviours than non handled piglets after the conditioning (ReuPC3). This was also
558 observed during the sessions of additional positive contacts of the conditioning: positive handled
559 piglets started with a higher score associated with investigation than non handled piglets (CondPC2)
560 and it held over the conditioning. Piglets that were positively handled at weaning also expressed a
561 higher mobility than non handled piglets (CondPC3). These observations may be interpreted as an
562 expression of natural foraging and disinterest from human contact, which may be a sign of positive
563 welfare (Weerd & Day, 2009). In addition, this could also be interpreted in terms of attachment to
564 the human. Indeed, attachment to a human may facilitate exploration of novel environments or
565 objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may
566 provide an environment secure enough for the piglets to explore their environment in the presence of
567 the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al.,
568 2009).

569 Overall, the behavioural monitoring showed that two minute sessions of positive additional contacts
570 per day are sufficient to increase proximity to a human to similar levels as when piglets were
571 previously familiarised for 2 weeks, even when piglets experienced social isolation. But it did not
572 allow the non handled piglets to express natural exploratory behaviours as the positively handled
573 piglets. We may be able to hypothesize a sequential establishment of a positive HAR over time:
574 firstly with a decrease of attentive state and an increase in proximity and accepted contacts, and
575 secondly with a disinterest of human contacts and the expression of natural foraging behaviour. The
576 latter may require a higher exposure time.

577 ***Links between vocal expression and positive HAR***

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

standard reunion test before and after conditioning. This enables us to raise two possible hypotheses. We then use the results of the sessions of additional positive contacts of the conditioning to discuss theses hypotheses.

Human mere presence affects vocal expression according to previous experience

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

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

We showed that the proximity to the human changed the structure of piglets grunts and that proximity significantly interacted with the treatment and the conditioning time. Indeed, similarly to a previous study (Villain et al. 2020a, 2020b), during the standard reunion test (no contact from the human), piglets produced shorter and higher pitched grunts with an upshifted frequency spectrum when closer to the human. It was especially the case in non handled piglets, before the conditioning. These results may be linked to the reactivity to the human, non handled piglets being more reactive to the presence of a human than positively handled piglets. Indeed, before the sessions of the conditioning, positively handled piglets were habituated to a human interacting positively when present whereas non handled piglets were not, hence, during the first standard reunion test, when the human is present but do not interact with the piglet, positively handled and non handled piglets may have diverging expectations regarding the presence of the static and silent human. As positively

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

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

This test may allow us to suggest two potential non-exclusive hypotheses to explain why the effect of human proximity on grunt acoustic structure attenuates as the familiarity to the human increases. In a first hypothesis, we could think that this attenuation of acoustic flexibility is due to a decrease in reactivity to the human, which may be linked to a disinterest of human contacts and an increase in natural foraging behaviours. In another hypothesis, this attenuation of acoustic flexibility may be due to a violation of piglets expectations: because the human remains static during the test, this may inhibit vocal reactions to the proximity. The interpretation of the second type of human-piglet interactions below may allow us to address these hypotheses.

Providing rewarding additional positive contacts changes the structure of grunts

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

testing the existence of human directed vocalisations in pigs, as consequences of their socio communicative abilities.

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

We found that the identity of the human had effects on behavioural and vocal response scores. Piglets that were handled by the human 'AH' had higher values of behavioural proximity (ReuPC1) than piglets handled by the human 'AV' during reunion test after a period of isolation. This effect was not found during conditioning sessions. The effect of the human did not interact with the conditioning time, leading to the conclusion that the difference between the two experimenter may have establishment during the period of positive handling at weaning, prior to the conditioning. Additionally, when the human 'AH' was in the room, piglets produced grunts with a more upshifted frequency spectrum and a higher pitch than when the human 'AV' was in the room. If upshifted grunts may be a indicator of positive higher arousal, then we may conclude that 'AH' was more likely to trigger higher positive states than 'AV'. Interestingly, the human identity and the spatial proximity had different effects on piglets grunts during sessions of additional positive contacts but not when the human was static during the standard reunion test. Hence, it is possible that the way one human interacts (behavioural and vocally) with a piglet may be more or less effective at triggering positive emotions and thus modifications of grunt structure. Several evidence exists in the literature that pigs discriminate humans visual and auditory cues (Bensoussan et al., 2019; Brajon et al., 2015c). Pigs may also show behavioural changes hearing human voice (Bensoussan et al., 2020). We may question the efficiency of different human features to generate a positive HAR. In our study, both humans that interacted with the piglets wear exactly the same clothes and standardized their tactile interactions toward the piglets before starting the study, and agreed on the rhythm and types of sounds (words, intonation) to use, to minimise generating variability although no systematic controls of the human behaviour or spectral feature of voices were performed here. It thus remains unclear whether experimenters interacted differently or if they were initially perceived differently by piglets. Our results show that the identity of the human may modulate piglet proximity and vocal behaviour but the design of this experiment does not allow to find the causes of these observations (behaviour, voice characteristics, or even odour profile). Thus, more studies of human features that are most likely to generate a positive HAR are needed and may be of interest regarding animal welfare. In addition, studying human-piglet relationship in a more systematic way, as in other domestic species, for example the play behaviour in dogs (Horowitz & Hecht, 2016) or the pet directed speech (Jeannin et al., 2017; Lansade et al., 2021), may shed light on the evolution and converging strategies of interspecific relationships. However, the influence of human identity did not modify the general

outcomes of our study, but only decreased some effects, suggesting that this variability does not modify the main results, but should be considered in future studies.

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

Authors contributions

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

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Data availability

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

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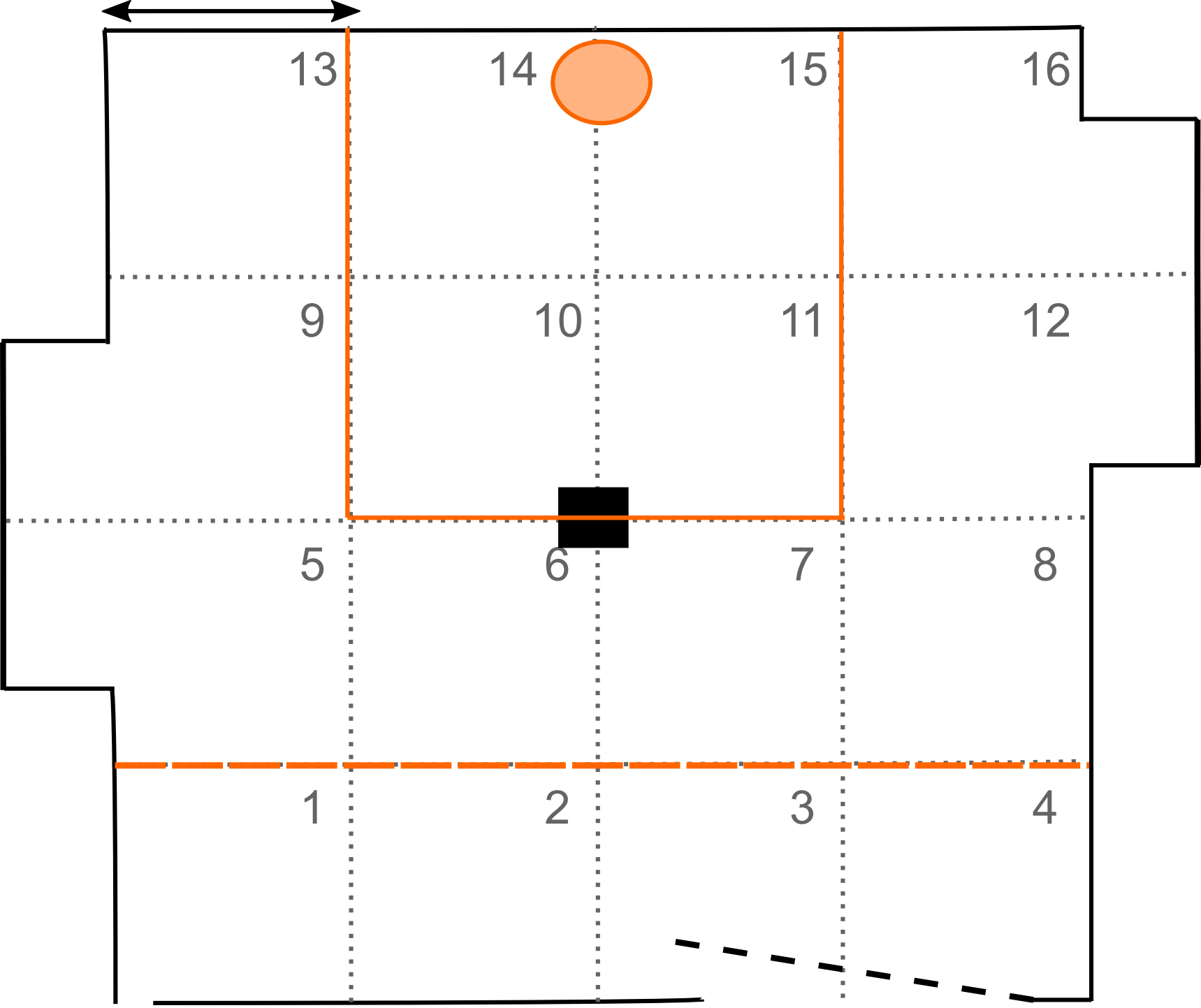


65.5cm

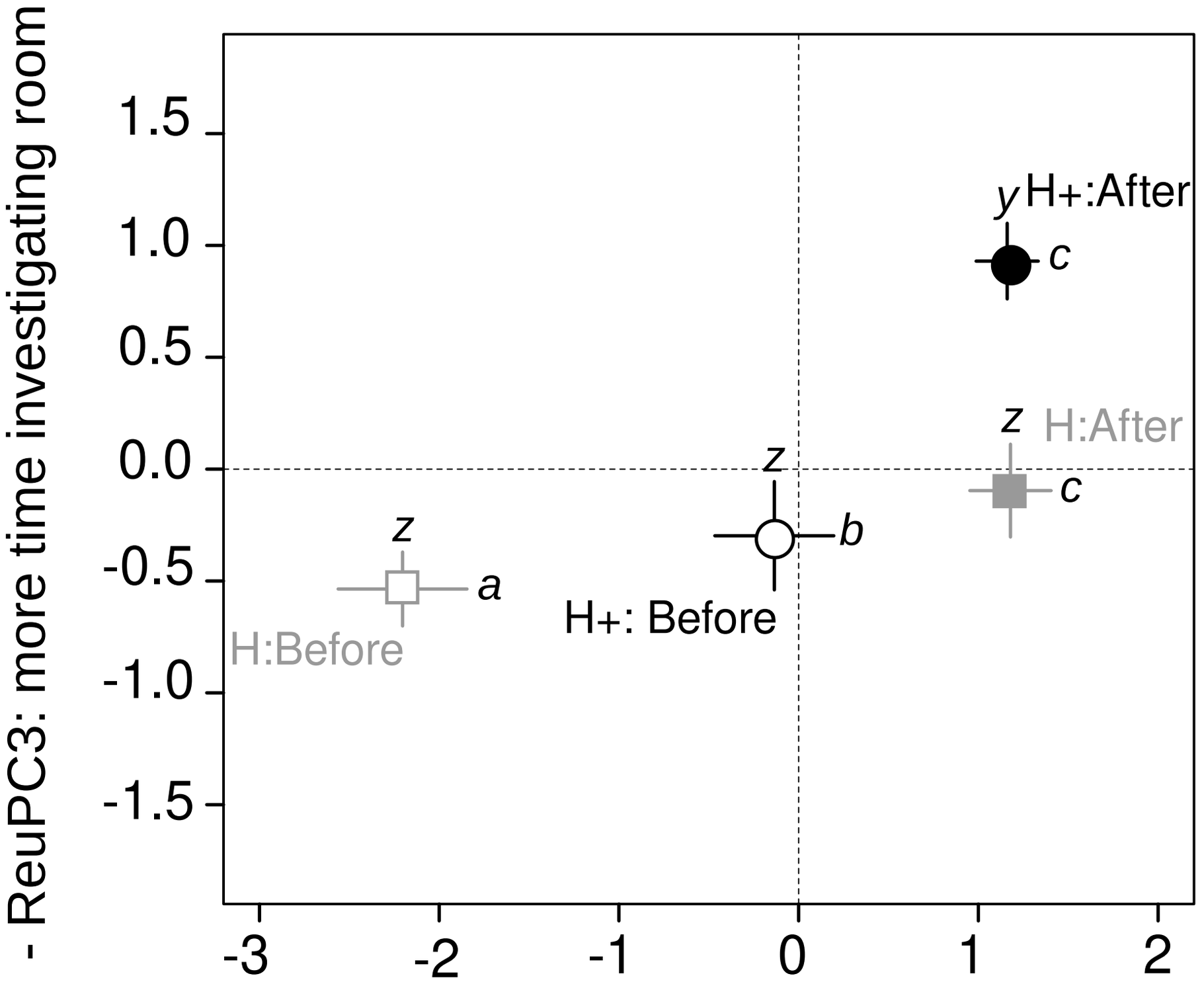
Location of the microphone

Virtual zones for behavioural scoring

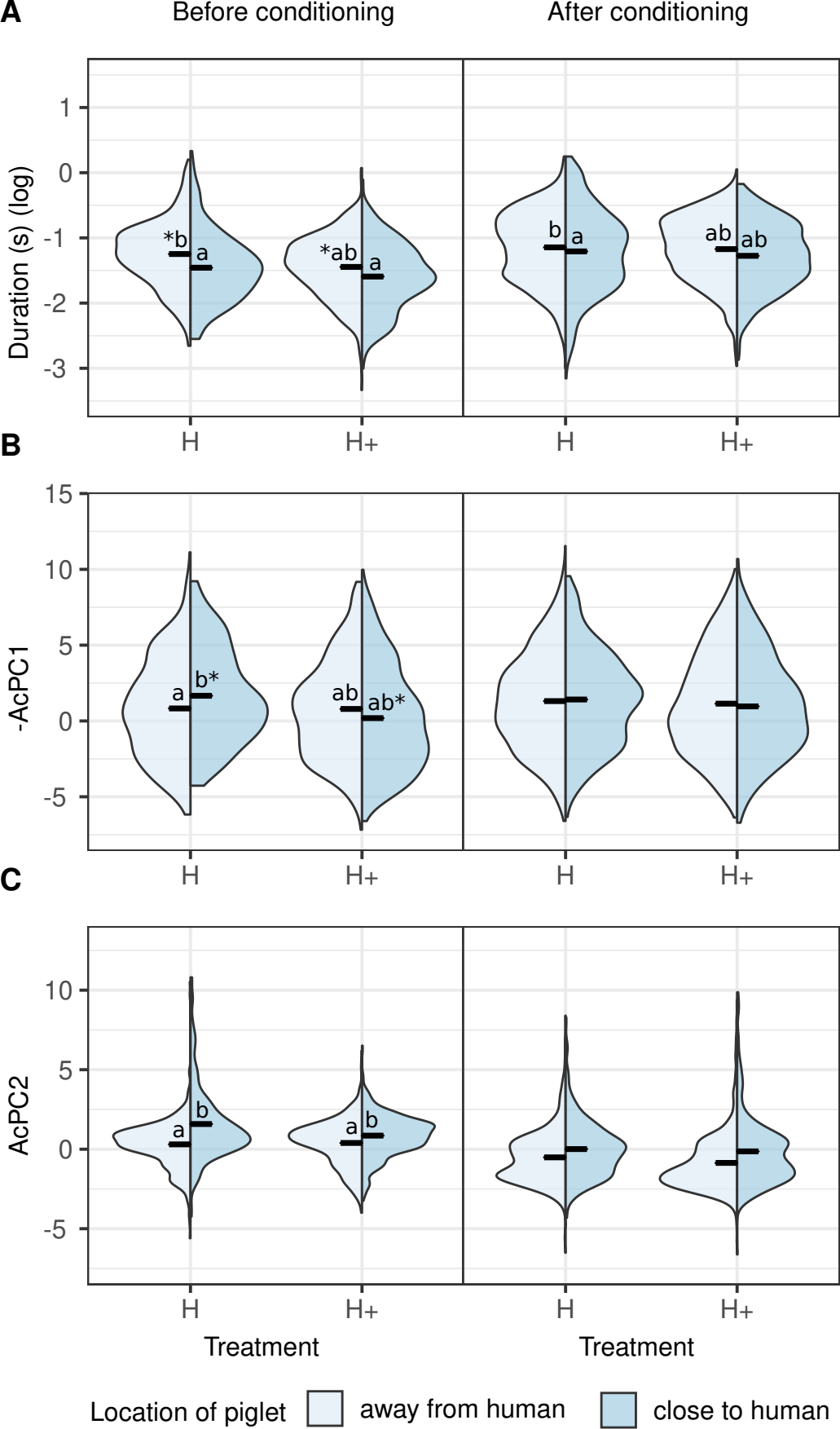
Location of the human

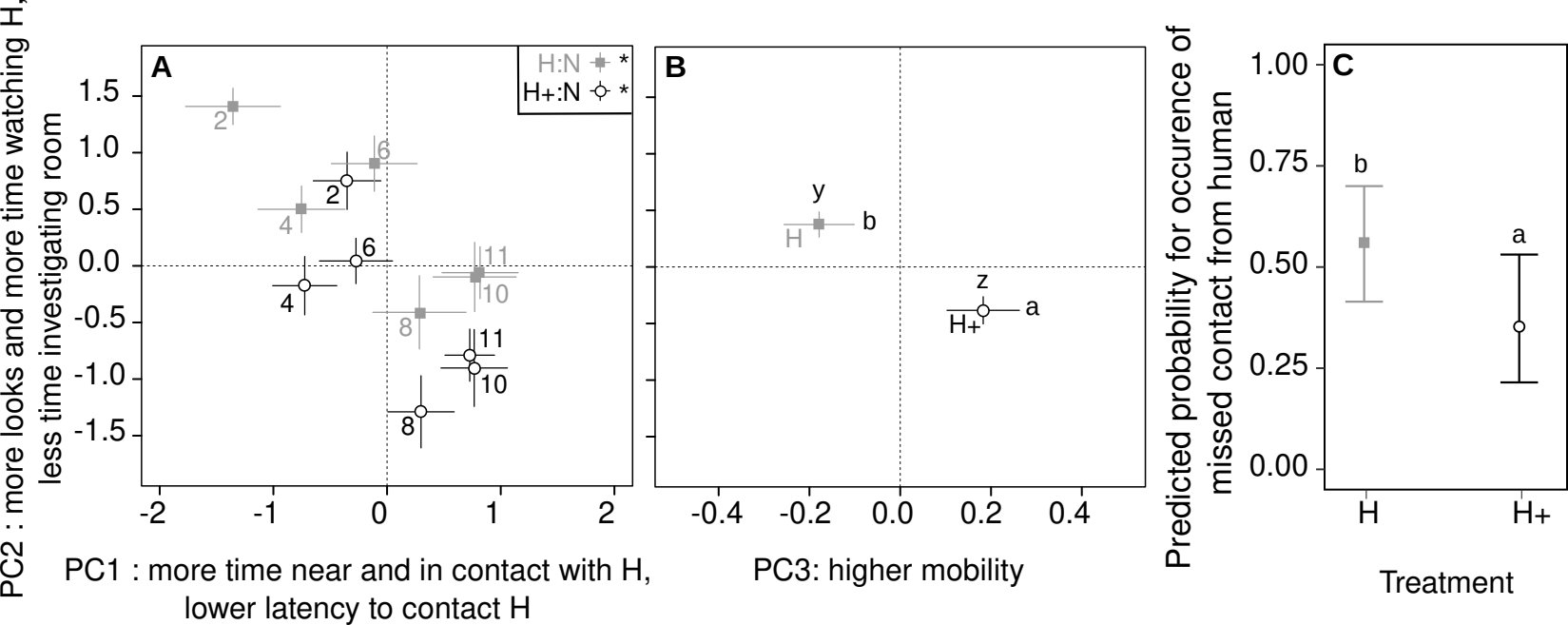
Proximal zone

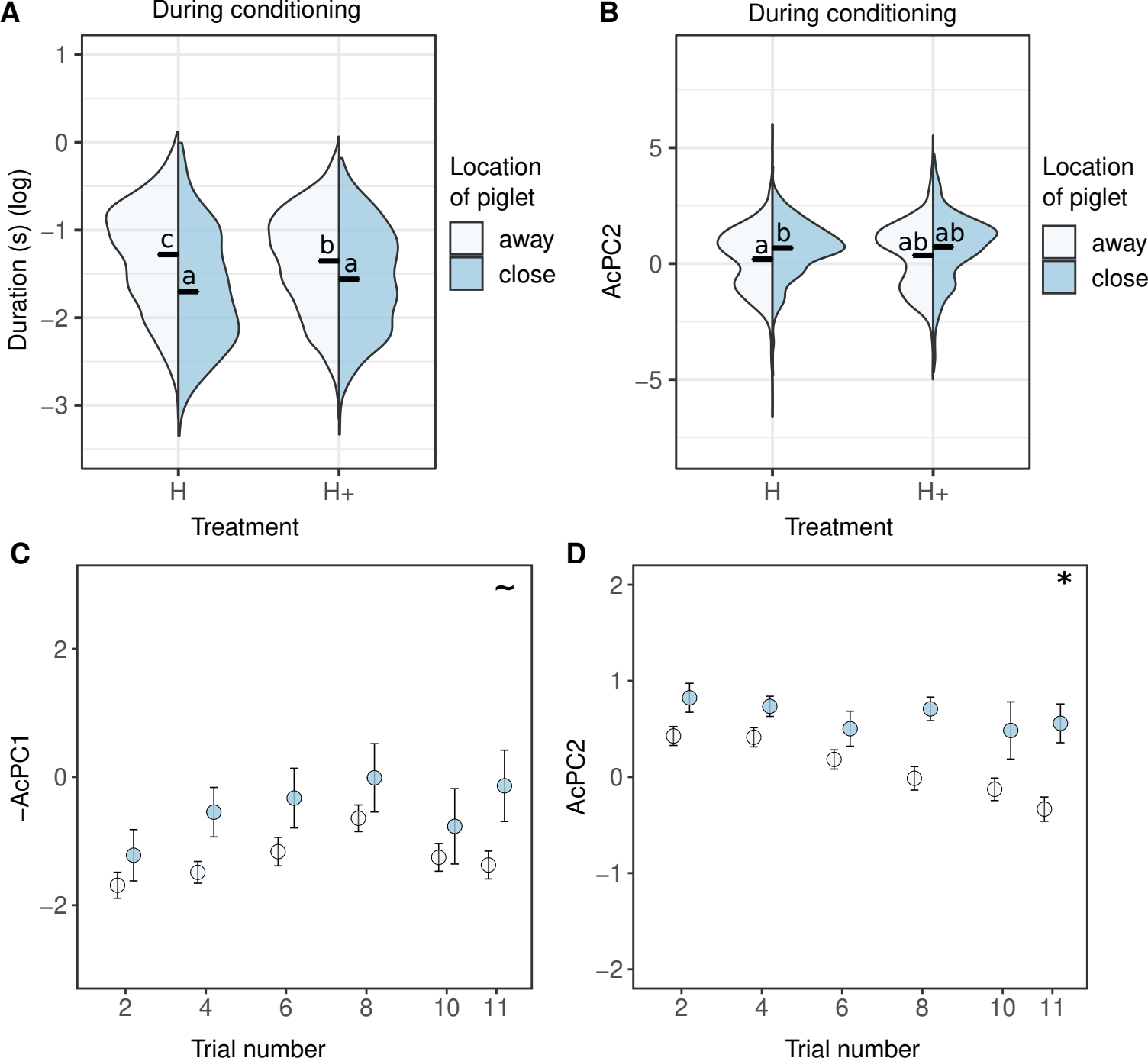
Distal zone

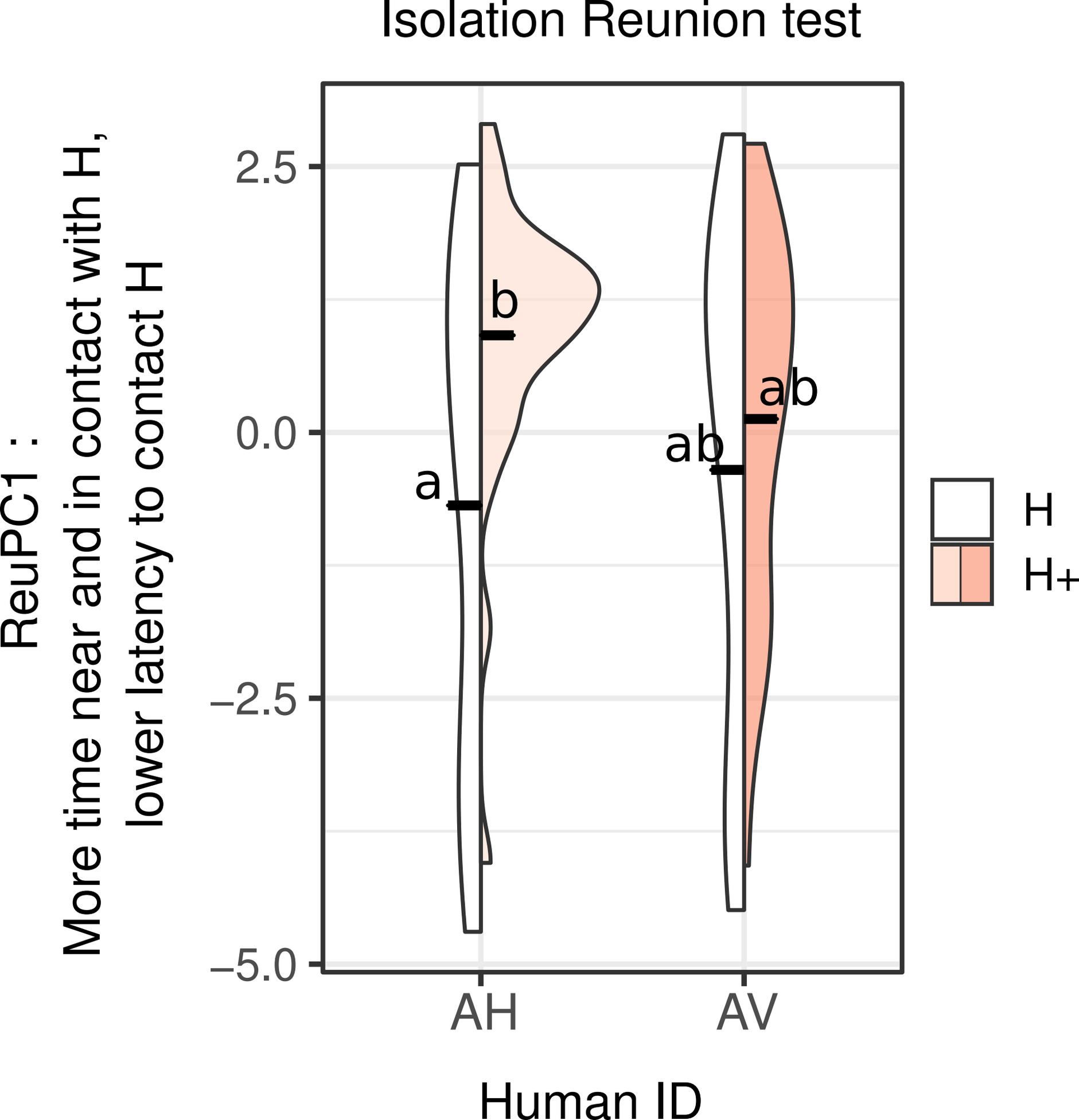


ReuPC1: lower latency to contact H,
more approaches and more time closer to H,
less time watching H, more investigation of H


A
Before conditioning
After conditioning
Duration (s) (log)
1
0
-1
-2
-3
*b
a
*ab
a
b
a
ab
ab
H
H+
H
H+
B
-AcPC1
15
10
5
0
-5
a
b*
ab
ab*
H
H+
H
H+
C
AcPC2
10
5
0
-5
a
b
a
b
H
H+
H
H+
Treatment
Treatment
Location of piglet
away from human
close to human


A
H:N *
H+:N *
PC2 : more looks and more time watching H, less time investigating room
PC1 : more time near and in contact with H, lower latency to contact H
B
PC3: higher mobility
C
Predicted probability for occurence of missed contact from human
Treatment
H
H+


A
During conditioning
Duration (s) (log)
1
0
−1
−2
−3
c
a
b
a
H
H+
Treatment
Location of piglet
away
close
B
During conditioning
AcPC2
5
0
−5
a
b
ab
ab
H
H+
Treatment
Location of piglet
away
close
C
−AcPC1
2
0
−2
~
2
4
6
8
10
11
Trial number
D
AcPC2
2
1
0
−1
−2
*
2
4
6
8
10
11
Trial number


Isolation Reunion test
ReuPC1 :
More time near and in contact with H,
lower latency to contact H
2.5
0.0
−2.5
−5.0
b
a
ab
ab
AH
AV
Human ID
H
H+