

Manuscript – Submission 1 – piglets anticipation grunts

1 **Title**

2 Piglets vocally express the anticipation of (pseudo)-social contexts in their grunts

3 **Authors**

4 A.S. Villain^{1*}, A. Hazard¹, M. Danglot¹, C. Guérin¹, A. Boissy², C. Tallet^{1*}

5 [1] PEGASE, INRAE, Institut Agro, 35590, Saint Gilles, France

6 [2] INRAE, Université Clermont Auvergne, Vetagrosup, UMRH, 63122 St-Genès
7 Champanelle, France

8

9 Corresponding authors :

10 * avelyne.sylvie.villain@gmail.com, celine.tallet@inrae.fr

11 **Key words**

12 Bioacoustics, welfare, human-animal relationship, positive emotions, communication

13

14 **Abstract**

15 Emotions not only arise in reaction to an eliciting event but also while anticipating it, making
16 this context a way to assess the emotional value of events. Anticipatory studies have poorly
17 considered vocalisations whereas they carry information about the emotional state. We studied
18 the grunts of piglets that anticipated two (pseudo)social events known to elicit positive emotions
19 more or less intense: arrival of a familiar conspecifics and arrival of a familiar human. Both
20 time and spectral features of the vocal expression of piglets differed according to the emotional

Manuscript – Submission 1 – piglets anticipation grunts

21 context. Piglets produced low-frequency grunts at a higher rate when anticipating conspecifics
22 compared to human. Spectral noise increased when piglets expected conspecifics, whereas the
23 duration and frequency range increased when expecting a human. When the arrival of
24 conspecifics was delayed grunts duration increased, while when the arrival of the human was
25 delayed spectral parameters were comparable to those during isolation. This shows that vocal
26 expressions in piglets during anticipation are specific to the expected reward and to the time
27 duration between signal and the delivery of the reward. Vocal expression (time and spectral
28 features) is thus a good way to explore emotional state of piglets during anticipation of
29 challenging events.

30 **Introduction**

31 Animal behaviour is driven by the motivational system¹. Animal's emotions are important
32 feedback mechanisms for modulating the activity of this system. One way to assess the
33 emotional value of an event is to measure the anticipatory activity before the event. Indeed,
34 emotions not only arise in reaction to the challenging event, but also during anticipation of this
35 expected event². Anticipation is goal directed and occurs during the appetitive phase of
36 behaviour³, before the consummatory phase. Anticipatory behaviour towards a positive event
37 is adaptive since it is associated with the motivational system that directs the animal from an
38 aversive state (e.g. hungry) to a reinforcing state (e.g. food acquisition; see Spruijt *et al.*¹). It is
39 suggested that promoting positive anticipation is a way to enhance the quality of life of animals
40 that are under the responsibility of humans⁴.

41 During anticipation of a positive event, animals are motivated for the event that will arise, and
42 are thus more likely to pay attention to stimuli that are signalling the event itself³. Anticipation
43 has been used to evaluate the sensitivity and motivation to different events supposed to be

Manuscript – Submission 1 – piglets anticipation grunts

44 positive like food reward, social contact, and play^{3,5-7}. It may also be used to evaluate cognitive
45 judgement bias that are consecutive to a long terms emotional experience⁸.

46 Anticipation of social contact and play is expressed by an increase of the time spent in a
47 compartment where the given social reward is expected to arrive in rats⁵, and an increase of
48 activity in rats, silver foxes, pigs, horses and lambs^{5,7,9-11}. Comparing the behavioural
49 anticipation responses to different events allows evaluating both the relative valence and the
50 intensity of the emotion associated to the expected events. For instance, lambs express a higher
51 elevation of the activity and more behavioural transitions before food than before play,
52 suggesting that food reward is a more intense positive event⁷. In hens, behavioural anticipation
53 is different according to the quality of the food reward⁶. In other cases, the increase of the level
54 of activity is not specific to the quality of the anticipated event. For instance, in a study in pigs,
55 the level of locomotor activity during anticipation of a positive (food) and a negative
56 (frightening event) events do not differ¹². In silver foxes, differences in anticipatory behaviour
57 before different food rewards are shown in the posture of the ears, but not in the level of
58 activity¹⁰. This suggests anticipatory behaviour may be event-specific and species-specific
59 e.g.¹³. Thus specific anticipatory behavioural activations, i.e. activity and spatial location, may
60 not be sufficient to highlight differences in anticipatory behaviour in all cases.

61 In addition, vocalisations may be interesting measures to explore emotional content of the
62 anticipation phase. Indeed, vocalisations have an emotional content in many species¹⁴.
63 However, vocalisations have been poorly included in the anticipatory behaviour ethograms
64 until now. One exception are the rats' frequency modulated ultrasonic vocalisations (50kHz)¹⁵.
65 In horses, low-pitched vocalisations (i.e. nickers) are proposed by Peters *et al.*¹¹ as expressions
66 of positive anticipation but the authors were not able to score them in their study. In pigs, high-
67 frequency vocalisation are suggested to be a good indicator of the emotional state during
68 anticipation of different events¹². The probability that a pig makes high-frequency vocalisations

Manuscript – Submission 1 – piglets anticipation grunts

69 is higher before negative than positive events¹². Pig are a good candidate to study vocalisations
70 during anticipation because the variability in vocal expressions according to emotions has been
71 reported both according to the valence and the arousal dimensions of emotions¹⁶⁻¹⁹. Thus
72 vocalisations quality, especially of grunts, would be a good indicator to evaluate anticipation in
73 pigs.

74 In the present study, we wanted to measure the vocal expression in piglets during the
75 anticipation of events with different emotional values. In the farming context, pigs may
76 experience various kinds of (pseudo)social events. Pigs being social animals the presence of
77 familiar conspecifics has a highly positive valence²⁰. Pigs also experience interactions with
78 humans and may develop positive relationship with them after a period of positive reinforcing
79 interactions, such as brushing and calmly speaking, compared to control animals²¹⁻²³. Positive
80 anticipation of human contact is possible in captive non domestic animals²⁴, and we tested this
81 in pigs.

82 The aim of this study was to test whether piglets reared in-group could vocally express
83 anticipation of arrival of social partners and arrival of a familiar human caregiver and if
84 vocalisations were different according to the social characteristics of the reward. We first
85 conditioned piglets to associate a visual and acoustic signal to the arrival of familiar
86 conspecifics and another signal to the arrival of a familiar human caregiver. Half of the piglets
87 had previously received additional positive contacts with the human prior to the conditioning,
88 leading to two groups of piglets with different degrees of familiarity toward the human. To
89 complete the investigation of emotional values of the anticipated event, we carried out a final
90 test delaying the arrival of the expected partner. We measured both their behavioural and vocal
91 activity, as well as the acoustic structure of their grunts, during the signal, e.g. the anticipatory
92 phase, and then after the signal when the reunion was delayed. We hypothesized that expecting
93 familiar conspecifics has a positive valence and induces a high arousal state for all piglets,

Manuscript – Submission 1 – piglets anticipation grunts

94 compared to expecting a familiar human, even considering the quality of his familiarity. If vocal
95 signals reflect emotional states, we expect them to have a different signature when anticipating
96 conspecifics compared to a human. If having received additional contacts with the human prior
97 to the conditioning modifies the emotional state of piglets, we expect a different anticipatory
98 vocal signature between groups that had or had not received additional care.

99 **Results**

100 ANTICIPATORY BEHAVIOUR DIFFERS BETWEEN PIGLETS EXPECTING THE ARRIVAL OF
101 A HUMAN OR CONSPECIFICS

102 One trial was separated in five phases, tested as factors: before the signal was broadcasted
103 (phase -1), while the signal was broadcasted, i.e during the anticipation phase (phase 0) and
104 when the arrival of the partner was delayed of 1.5 minutes, which was segmented in three 30-
105 second phases (1, 2 and 3).

106 *Table 1: Loadings of linear discriminant functions 1 and 2 (respectively LD1 and LD2) of the behavioural analysis*
107 *of anticipation in piglets trained to expect the arrival of familiar conspecifics or a familiar human. In lines, the*
108 *parameters used to build the functions and in columns their respective loadings on the first two functions. Note*
109 *that this table concerns the data from phase ‘-1’ (before the signal) and phase ‘0’ (during the signal) and that a*
110 *factor taking into account the partner, the phase and the treatment was used to discriminates the groups. These*
111 *loading were then used to project the data gathered during the violation of expectations test (trial 12) and statistics*
112 *were run on LD1 and LD2 after projection as two behavioural scores.*

Behavioural scores	LD1	LD2
Total time spent freezing	0.062	-0.181
Averaged duration spent per zone	-0.044	0.438
Number of zones explored	0.226	0.134
Total time spent watching upcoming partner's door	-0.224	0.356

Manuscript – Submission 1 – piglets anticipation grunts

Number of time watching upcoming partner's door	-0.188	0.664
Total time spent in zones near upcoming partner's door	-1.083	-0.708

113

114 Behavioural parameters were used to build two scores using a multivariate analysis carried out
115 in two steps. A linear discriminant analysis was first computed on a subset of data containing
116 the first two phases of the trial, e.g. before (phase -1) and during the signal (=phase 0, or
117 anticipation phase). Two behavioural scores corresponding to the first two linear discriminant
118 functions (LD1 and LD2) were thus built. On the remaining dataset, containing the last three
119 phases (after the signal: named 1, 2, and 3), a projection was computed on LD1 and LD2,
120 allowing to test for differences between the phases in the 2D behavioural space.

121 The first behavioural score (LD1) was negatively correlated with the time spent near the
122 upcoming partner's door and the time spent watching this door and positively correlated with
123 the number of zones explored (table 1). Statistics showed a significant interaction between the
124 phase of the trial and the partner ($X^2_4 = 13.9$, $p = 0.008$, figure 1A, significance letter from a to
125 d). During trials of anticipation of the human partner, the signal led to a significant decrease of
126 LD1 compared to the initial phase (H partner, phase -1 vs. 0, T.ratio = 5.97, $p < 0.001$). After
127 the signal, while the arrival of the partner was delayed, LD1 increased and then remained stable
128 (H partner, phase 0 vs. 1: T.ratio = -1.64, $p = 0.83$, 0 vs. 2: T.ratio = -3.88, $p = 0.004$, 0 vs. 3:
129 T.ratio = -3.82, $p = 0.005$); remaining at the same level as before the signal (H partner, phase -
130 1 vs. 1:2:3, $|T.ratio| < 2.62$, $p > 0.21$). During trials of anticipation of conspecifics, the signal
131 led to a significant decrease of LD1 compared to the initial phase (C partner, phase -1 vs. 0,
132 T.ratio = 7.33, $p < 0.001$). After the signal, LD1 did not change while the arrival of the partner
133 was delayed (C partner, phase 0 vs. 1:2:3, $|T.ratio| < 2.7$, $p > 0.18$), but tended/were to be
134 different than during the initial phase (C partner, phase -1 vs. 1, T.ratio = 2.5, $p = 0.25$; phase -
135 1 vs. 2, T.ratio = 3.1, $p = 0.06$; phase -1 vs. 3, T.ratio = 5.1, $p < 0.001$). Prior to any signal, LD1

Manuscript – Submission 1 – piglets anticipation grunts

136 differed depending on the type of partner (phase -1, C vs. H, T.ratio = -14.74, $p < 0.001$). No
137 interaction between the phase of the trial and the additional care treatment (H versus H+) was
138 found ($X^2_4 = 0.69$, $p = 0.95$) and no main effect of the treatment was found ($X^2_1 = 0.13$, $p = 0.72$).
139 The second behavioural score (LD2) was negatively correlated with the time spent near the
140 upcoming partner's door and the time spent freezing and positively correlated with the time
141 spent watching the upcoming partner's door, the number of times watching the upcoming
142 partner's door and time spent per zone (table 1). Statistics showed a significant interaction
143 between the phase of the trial and the partner ($X^2_4 = 49.1$, $p < 0.001$, figure A1, significance
144 letter from x to z). During trials of anticipation of the human partner, the signal lead to a
145 significant increase of LD2 compared to the initial phase (H partner, phase -1 vs. 0, T.ratio =
146 11.29, $p < 0.001$). After the signal, LD2 significantly decreased while the arrival of the partner
147 was delayed (phase 1) and then remained stable (H partner, phase 0 vs. 1:2:3 |T.ratio| < 6.37 , p
148 < 0.001), at the same level as before the signal (H partner, phase -1 vs. 1:2:3, |T.ratio| < 3.20 , p
149 > 0.05). Such effects were not significant for trials of anticipation of conspecifics, for which
150 only a trend was found toward an increase of LD2 during the anticipation phase (C partner,
151 phase -1 vs. 0, T.ratio = -3.01, $p = 0.08$). No difference was found between the anticipation
152 phase and the phase while the arrival of the partner was delayed (C partner, phase 0 vs. 1:2:3
153 |T.ratio| < 1.48 , $p > 0.90$). Before the signal we found no effect of the upcoming partner on LD2
154 (phase -1, C vs. H, T.ratio = 1.10, $p = 0.98$). No interaction was found between the phase of the
155 test and the treatment ($X^2_4 = 2.59$, $p = 0.63$) and no main effect of the treatment was found
156 ($X^2_1 = 2.26$, $p = 0.61$; figure 1A, significance letter from x to z).

157 LD1 and LD2 were mainly explained by the location of the piglet in the experimental room,
158 and piglets significantly spent more time near the conspecifics' door (see stats for LD1). So
159 differences between LD1 prior to the emission of any signal could be explained by either
160 location biases in the test room or the expression of a preference toward the conspecific door.

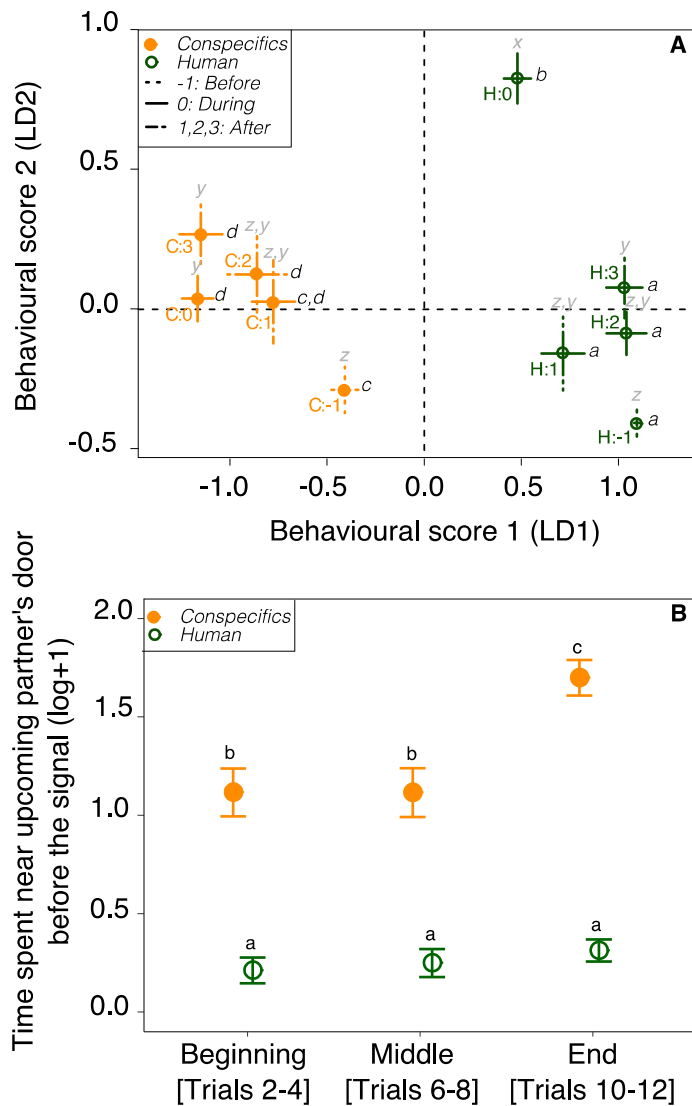
Manuscript – Submission 1 – piglets anticipation grunts

161 We thus tested the effect of the trial number (grouping trials at the beginning, the middle and
162 the end of the experiment, figure 1B) on the time spent near the upcoming partner's door. If
163 piglets expressed a preference toward the conspecific door along the conditioning, the
164 interaction between the trial number and the partner should be significant. Statistics showed a
165 significant interaction between the partner and the conditioning trial number ($X^2_2 = 11.96$, $p =$
166 0.003): although piglets spent more time near the conspecifics' door than near the human's
167 door, independently from the on-going partner, piglets increased their time near the
168 conspecifics' door at the end of the conditioning (C partner, middle vs. end of the conditioning,
169 $T.ratio = 4.19$, $p = 0.001$), but did not increase their time spent near the human door (H partner,
170 pairwise tests between all trial factors, $|T.ratio| < 0.8$, $p > 0.97$).

171 For full statistical report, refer to tables S1 and S3 of the supplementary material.

172

Manuscript – Submission 1 – piglets anticipation grunts



173

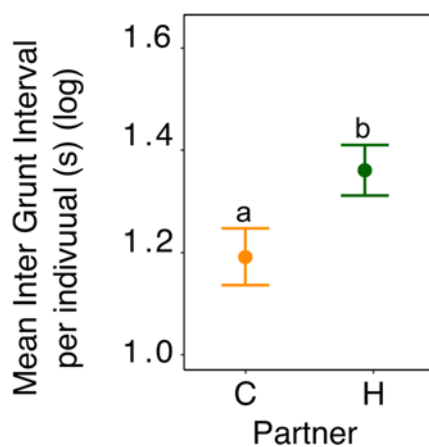
174 *Figure 1: Behavioural responses of piglets to the anticipation of the entrance familiar conspecifics (C, filled*
 175 *orange points) or a familiar human (H, empty green points). Points and bars represent mean ± se per group. A:*
 176 *behavioural space with LD1 and LD2 behavioural scores showing the significant interaction between the phase*
 177 *(-1, 0, 1, 2 and 3) of the test and the type of partner. Phase -1 corresponds to the time before the broadcasting of*
 178 *a signal, phase 0 corresponds to the time during the signal and phases 1, 2, and 3 are 30 second segments during*
 179 *the violation of expectation period (90 seconds in total). Letter from a to d and x to z show significant differences*
 180 *on LD1 and LD2 respectively. B: Time spent near upcoming partner's door before the broadcasting of any signal*
 181 *(phase-1 only) along the conditioning (grouping trials to create three factors: beginning, middle and end). Letters*

Manuscript – Submission 1 – piglets anticipation grunts

182 shows significant differences between groups. All model anova tests, estimates and pairwise post hoc tests with
183 Tukey contrasts are available in tables S1 S2 and S3 respectively as supplementary material.

184 VOCAL DYNAMIC DIFFERS BETWEEN PIGLETS EXCEPTING A HUMAN OR CONSPECIFICS

185 The grunt rate, during the anticipation phase (phase 0), was tested using the mean individual
186 inter-grunt interval. The inter-grunt interval was significantly lower during anticipation of
187 conspecifics than during anticipation of human ($X^2_1=4.35$, $p = 0.037$, figure 2), without any
188 interaction with the treatment ($X^2_1=0.037$, $p = 0.85$, table S1).



189
190 *Figure 2: Vocal dynamic in piglets during the anticipation phase (phase 0). Mean \pm se inter-grunt interval per*
191 *individual per type of intra or interspecific partner. Different letters represent significant differences between*
192 *expected partners. All model anova tests, estimates and pairwise post hoc tests with Tukey contrasts are available*
193 *in supplementary tables S1, S2 and S3.*

194 THE ACOUSTIC STRUCTURE OF ANTICIPATORY GRUNTS DIFFERS BETWEEN PIGLETS
195 EXCEPTING THE ARRIVAL OF A HUMAN OR CONSPECIFICS, AND DEPENDS ON THE
196 DEGREE OF FAMILIARITY WITH THE HUMAN

197 The acoustic structure of 2270 grunts (see table S4 for data composition) was analysed using
198 the duration of the call and a built-in spectral score, i.e. the first linear discriminant function
199 built from nine acoustic parameters representative of the call spectrum (LD1, table 2). Similarly,
200 to the behavioural analysis, the spectral score LD1 was built using the first two phases of the
201 test (before and during the signal). Then for the last three phase, LD1 values were computed

Manuscript – Submission 1 – piglets anticipation grunts

202 after projection on the same linear discriminant analysis. In order to test for acoustic responses
203 to anticipatory signals, interactions between the phases of the test, the partner and the treatment
204 were tested using linear mixed effect models.

205 *Table 2: Loading of the first linear discriminant function (LD1) following the spectral analysis of grunts in piglets.*
206 *In lines, the parameters used to build the functions and in column their respective loadings on the first function.*
207 *The linear discriminant analyse was made with the data before and during the signal (phases -1a and 0). A factor*
208 *taking into account the partner, the phase and the treatment was used to possibly discriminate or not the groups.*

parameters	Loading on the first function
Mean	-1.442
Median	-0.161
Mode	0.198
Q25	0.034
Q75	0.308
Centroid	-1.442
SH	2.888
SFM	2.249
Entropy (H)	-2.817

209

210 *THE ACOUSTIC STRUCTURE OF ANTICIPATORY GRUNTS DIFFERS BETWEEN PIGLETS*
211 *EXCEPTING A FAMILIAR HUMAN OR CONSPECIFICS*

212 To test the effect of the social quality of the partner (conspecifics vs. familiar human), the
213 interaction between the phase of the trial and the type of partner was studied and was significant
214 regarding both the duration of the call and the spectral structure ($X^2_4 = 50.3$, $p < 0.001$, figure
215 3A and $X^2_4 = 63.5$, $p < 0.001$ figure 3D respectively).

Manuscript – Submission 1 – piglets anticipation grunts

216 Considering the duration of the grunts, during trials of anticipation of the human partner, grunt
217 duration was longer than before the signal (partner H, phase -1 vs. 0, T.ratio = -4.79, $p < 0.001$).
218 After the signal, during the phase while the arrival the partner was delayed, grunt duration
219 remained as high as during the anticipatory phase (partner H, phase 0 vs. 1:2:3, $|T.ratio| < 1.66$,
220 $p > 0.82$) and was higher than before the signal (partner H, phase -1 vs. 1:2:3, $|T.ratio| > 3.59$,
221 $p < 0.012$). During trials of anticipation of conspecifics, the signal did not affect grunt duration
222 (partner C, phase -1 vs. 0, T.ratio = 2.42, $p = 0.315$). After the signal, grunt duration was higher
223 during all the phases while the partner was delayed (partner C, phase 0 vs. 1:2:3, $|T.ratio| >$
224 7.32 , $p < 0.0001$). The duration of grunt differed between the type of partner during the
225 anticipation phase (phase 0, C vs. H, T.ratio = -8.29, $p < 0.001$) but were not different before
226 (phase -1, C vs. H, T.ratio = -0.14, $p = 1.00$) nor during the violation of expectation phase (phase
227 1:2:3, C vs. H, $|T.ratio| < 0.34$, $p = 1.00$).

228 Considering the acoustic spectral score LD1 (table 2), statistics showed a significant interaction
229 between the phase of the trial and the type of partner ($X^2_4 = 63.5$, $p < 0.001$, figure 3D). During
230 trials of anticipation of the human partner LD1 decreased during the anticipation phase
231 compared to the preceding phase (H partner, phase -1 vs. 0, T.ratio = 3.95, $p = 0.003$) and
232 increased after the signal, while the arrival of the partner was delayed (H partner, phase 0 vs. 1:
233 T.ratio = -3.74, $p = 0.007$, phase 0 vs. 2: T.ratio = -2.85, $p = 0.12$, phase 0 vs 3: T.ratio = -4.72,
234 $p < 0.001$) and returned to LD1 values measured before the signal (H partner, phase -1 vs. 1:2:3,
235 $|T.ratio| < 1.29$, $p > 0.96$). During trials of anticipation of the conspecifics, LD1 increased during
236 the anticipation phase compared to the previous phase (H partner, phase -1 vs. 0, T.ratio = -
237 3.97, $p = 0.003$) and decreased after the signal, while the arrival of the partner was delayed (C
238 partner, phase 0 vs. 1:2:3, $|T.ratio| > 4.90$, $p < 0.001$) and returned LD1 values measured before
239 the signal (C partner, phase -1 vs. 1:2:3, $|T.ratio| < 0.66$, $p = 1.00$). LD1 was significantly higher
240 during anticipation of the conspecifics than the human (phase 0, C vs. H, T.ratio = 9.00, $p <$

Manuscript – Submission 1 – piglets anticipation grunts

241 0.001), and there was no difference between the partner for the other phases (phases -1:1:2:3,
242 C vs. H, $|T.ratio| < 0.66$, $p = 1.00$).

243 *THE ACOUSTIC STRUCTURE OF ANTICIPATORY GRUNTS DIFFERS WITH THE DEGREE OF*
244 *FAMILIARITY TOWARD THE HUMAN*

245 To test the effect of the familiarity of the human partner, the interaction between the phase of
246 the test and the treatment was studied and was significant regarding both the duration of the call
247 and the spectral structure ($X^2_4 = 12.4$, $p = 0.015$ figure 3B, and $X^2_4 = 14.8$, $p = 0.005$ figure 3E).
248 Regarding grunt duration, in the H+ group (handled piglets), we found no difference in the
249 duration of grunts during the anticipation phase compared to the other (H+ group phase 0 vs. -
250 1:1:2:3, $|T.ratio| < 2.95$, $p > 0.094$) but grunts were longer after the signal, while the arrival of
251 the partner was delayed, than before the signal (H+ group phase -1 vs. 1:2:3, $|T.ratio| > 3.75$, p
252 < 0.007). In the H group, the duration of grunts increased after the signal, while the arrival of
253 the partner was delayed (group H, phase 0 vs. 1:2:3, $|T.ratio| > 3.38$, $p < 0.025$) but did not
254 differ between the anticipation phase and the preceding phase (group H, phase -1 vs. 0, $T.ratio$
255 $= 0.23$, $p = 0.55$). A significant interaction was also found between the type of partner and the
256 treatment, independently of the phase ($X^2_1 = 5.84$, $p = 0.016$, figure 3C): piglets from the H group
257 produced longer grunts than piglets from the H+ group during trials of anticipation of a human
258 (H partner, H vs. H+, $T.ratio = 2.69$, $p = 0.04$) but there was no difference during trials of
259 anticipation of conspecifics (C partner, H vs. H+, $T.ratio = 1.31$, $p = 0.56$).

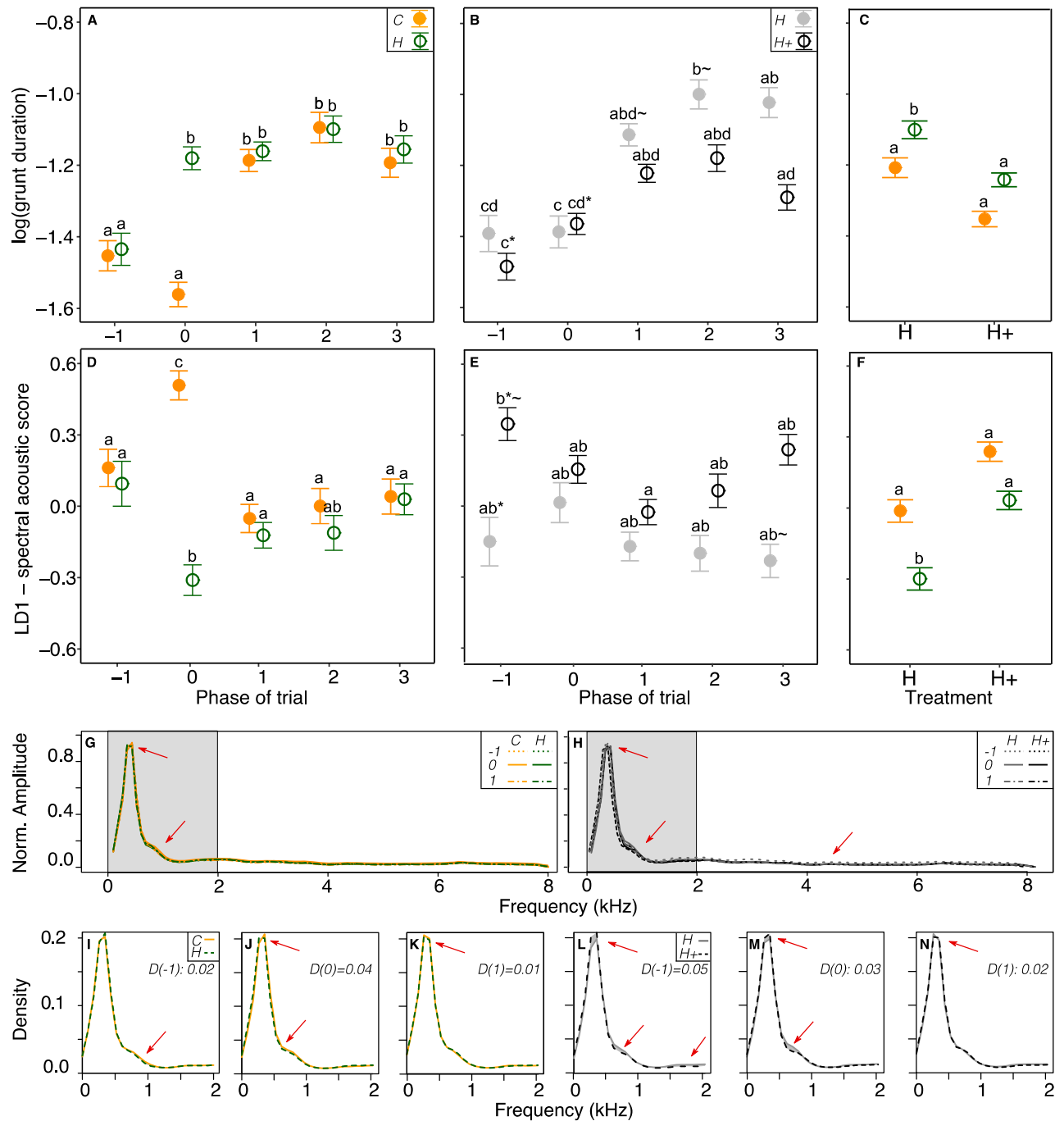
260 Regarding the spectral score LD1, in the H+ group, a significant decrease of LD1 was found
261 from the phase before the signal to the first phase while the arrival of the partner was delayed
262 (H+ group, phase -1 vs. 1, $T.ratio = 3.26$ $p = 0.038$) and all other comparisons between phases
263 did not differ (H+ group, all other phases, $|T.ratio| < 2.89$, $p > 0.11$). In the H group, no
264 difference was found in LD1 between all phases (H group, pairwise between all phases $|T.ratio|$
265 < 1.80 , $p > 0.73$), Within phases, no difference was found between treatments but LD1 in the

Manuscript – Submission 1 – piglets anticipation grunts

266 H+ group tended to be higher than LD1 in the H group before the signal was broadcasted (phase
267 -1, H vs. H+, T.ratio = -3.06, $p = 0.078$). All other comparisons between treatments were non-
268 significant (all phases, H vs. H+, $|T.ratio| < 2.69$, $p > 0.19$). A significant interaction was found
269 between the type of partner and the treatment, independently from the phase ($X^2_1=6.45$, $p =$
270 0.010 , figure 3F). LD1 of grunts produced by piglets from the H group in trials of anticipation
271 of the human partner were lower than in trials of anticipation of conspecifics (H group, C vs.
272 H, T.ratio = 4.02, $p < 0.001$), but not for piglets from the H+ group (H+ group, C vs. H, T.ratio
273 = 1.37, $p = 0.52$).

274 To illustrate spectral changes in grunts, mean spectra per group were represented in figure 3,
275 identifying the two types of significant interactions found with the phase of the test: type of
276 partner, (figure 3G, 3I-K) and treatment (3H, 3L-N). Mean spectra representing all frequency
277 range of grunt (0-8 kHz, figure 3G and 3H) were represented as well as zooms in on the specific
278 0-2 kHz range to identify the changes with arrows (figure 3I to 3N), in the latter zooms in, a
279 coefficient of difference between the mean spectra was calculated to have a better ideas of
280 difference between considered groups.

Manuscript – Submission 1 – piglets anticipation grunts



281

282 *Figure 3: Acoustic structure (mean ± se) of grunts in piglets depending on the type of partner [familiar conspecifics*

283 *(C, filled orange points) or human (H, empty green points)] and treatment [additional contacts group (H+, empty*

284 *black points) or minimal contact group (H, filled grey points). A. and B: evolution of grunt duration along phases*

285 *of the trials. C: grunt duration according to partner and treatment. D and E: evolution of spectral score LD1*

286 *along phases of the trials. F: Spectral score LD1 according to partner and treatment. Phases correspond to: before*

287 *the signal (phase -1), during the signal i.e anticipation phase (phase 0) and after the signal, i.e during the violation*

288 *of expectation phases (phases 1,2,3 of 30 seconds). Letters shows significant differences between groups. * and ~*

Manuscript – Submission 1 – piglets anticipation grunts

289 *symbols identify statistical trends between two groups. All model anova tests, estimates and pairwise post hoc tests*
290 *with Tukey contrasts are available in tables S1, S2 and S3 respectively as supplementary material. G-N:*
291 *representation of mean spectra per group (computed with ‘meanspec’ function, ‘seewave R package, wl=512,*
292 *overlap = 50%). Each line represents a mean over all the grunts extracted in a specific phase and/or treatment/type*
293 *of partner group. Due to extremely low variability in the spectrum per group, standard errors of the mean of all*
294 *spectra are not visible on the plots. The number of grunts used per group is available table S4 (56<N<241,*
295 *median=101 grunts on a total of 2270). Arrows indicate where the changes are the strongest. I-N: zooms in on the*
296 *0-2kHz frequency range, for which the coefficient $D(\text{phase})$ correspond to a metric of spectral dissimilarity*
297 *($0 < D < 1$, computed with ‘diffspec’ function, ‘seewave’ R package).*

298

299 **Methods**

300 ETHICAL NOTE

301 Experiments were performed under the authorization no. APAFIS#17071-
302 2018101016045373_V3 from the French Ministry of Higher Education, Research and
303 Innovation; and were in agreement with the French and European legislation regarding
304 experiments on animals.

305 SUBJECTS AND HOUSING CONDITIONS

306 Sixty weaned female piglets (in two replicates), *Sus scrofa domesticus*, bred from crosses
307 between Large White and Landrace females and Piétrain males were used for this study from
308 28 to 62 days after birth. Animal housing and experiments took place at the experimental unit
309 UEPR (UE 1421, INRAE France).

310 One piglet was removed in the middle of the experiment due to health issues independent from
311 the experiment. Piglets from the same litter and having similar weight (<1 kg difference) were
312 housed by three in a 1.2 x 1.3m pen on plastic duckboard and panels visually isolated pens. One

Manuscript – Submission 1 – piglets anticipation grunts

313 chain per pen was used for enrichment. Food and water were available *ad libitum*. Artificial
314 lights were turned on from 8:00 to 17:00 and temperature was maintained between 26 and 27
315 °C. Two identical rooms were used (5 pens per room per replicate).

316 EXPERIMENTAL TREATMENT: HUMAN ADDITIONAL CONTACTS

317 Two experimental treatments were generated as follows:

- 318 • A group with minimal human contact, H group: Control piglets from 10 rearing pens
319 received the minimal amount of daily contact with a stockperson (a 1.70m high male) required
320 for feeding, cleaning and health checking. The stockperson wore dark green shirt and pants with
321 brown shoes.
- 322 • A group with additional human contacts, H+ group: Animals from the 10 other rearing
323 pens received, in addition to daily care given by the stockperson as for H group, 29 sessions of
324 additional human gentle tactile contact from one of the two experimenters (both women, both
325 between 1.70-1.73, balanced number of pens attributed). The experimenters wore the same
326 overalls and boots each time they interacted with the pigs; i.e. blue overalls and dark green
327 boots. The handling procedure, using gentle tactile contacts, was similar to Tallet *et al.*²¹. Those
328 additional contacts were given from the day of weaning until day 39, with three sessions per
329 day (with a two hour break in between) except at weekends. The order of the pen was balanced
330 across days. We confirmed that the additional human contact treatment (H+) induced a positive
331 attraction toward the human in a standard human-piglet reunion test (supplementary material,
332 fig. S1).

333 TWO-WAY ASSOCIATIVE LEARNING AND INDUCTION OF ANTICIPATION

334 Pen piglets were habituated to the test room for 10 minutes, two days before the start. The
335 conditioning took place between day 42 and 62 after weaning, lasted twelve days, with two
336 trials per day with at least three hours between trials of the same day.

Manuscript – Submission 1 – piglets anticipation grunts

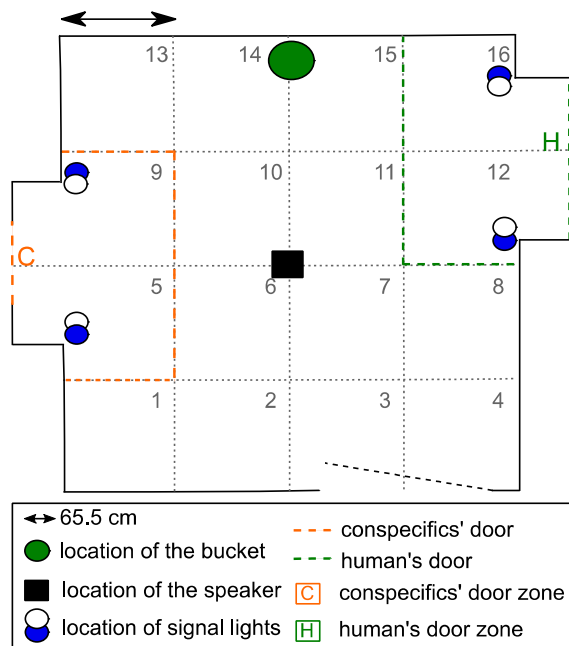
337 *(Pseudo) social events*

338 All piglets were individually trained to learn to associate two different signals with the arrival
339 of two different (pseudo)-social partners for 2 minutes: either three pen mates (partner =
340 Conspecifics) or a familiar human (partner = Human). When entering the room, the human sat
341 on a bucket and interacted with the piglet, in the same way she interacted with them during the
342 taming phase. For piglets from the H+ group, the human was already familiar (same as the
343 taming phase) whereas for piglets from the H group, the human was unfamiliar and became
344 familiar along the conditioning.

345 *Associative learning signals*

346 Associative learning stimuli were chosen to facilitate learning since the aim was not to test
347 learning abilities but the way piglets would anticipate the reunions. One signal announcing the
348 entrance of a partner combined one visual and one auditory stimulus ²⁵: visual stimuli were
349 lights (blue or white) lighting on nearby door and auditory stimuli were tones (296 Hz or 3100
350 Hz, broadcasted from a speaker (Mipro MA-100su, Mipro Electronics Co, Taiwan). Four visio-
351 auditory combinations were thus built and their occurrences were balanced across all
352 experimental piglets.

Manuscript – Submission 1 – piglets anticipation grunts



353

354 **Figure 4: Schematic of the test room.** The acoustically isolated room contained three doors: the human's door (H on
355 the right), the conspecifics' door (C on the left), and the entrance door (at the bottom), which remained located
356 this way during the entire experiment. A speaker, in the center of the room and at 1m high from the ground
357 broadcasted a 2sec signal, associated to the upcoming partner. Blue and white lights, around the partner's door
358 were used as visual signal, either blue or white (from two to 20 seconds) announcing the entrance of the partner.
359 When the human entered the room, they would bring a bucket and sit for two minutes bringing additional care to
360 the tested piglet. For behavioural analyses, the room was separated in 16 zones to allow quantifications of mobility
361 and location in the room as well as partner door zones (either C or H).

362 Associative learning trials

363 Twenty-four trials were run by piglet; 12 with each partner. For each trial, the target piglet
364 entered the experimental room and remained alone between 10 to 30 seconds to avoid
365 habituation to the start of the signal (phase -1) before the signal started and lasted between two
366 and 20 seconds (phase 0, anticipation). After the end of the signal, the partner entered the room.
367 Piglets from the same pen were tested one after the other and the order was balanced from one
368 trial to the other to avoid confounding effect of the order within one pen. The order of the pens
369 was balanced from one day to the other to avoid confounding effect of the period of the day.
370 Piglets were reunited once with each of the possible partner each day (balancing between the

Manuscript – Submission 1 – piglets anticipation grunts

371 morning and the afternoon), except on days 6 and 8 for which they were reunited the same
372 partner in the morning and the afternoon trials to avoid habituation to alternating reunions. This
373 design was inspired by Reimert *et al.*²⁵.

374 *Inducing and testing anticipation*

375 To generate an anticipatory phase (phase 0) prior to the arrival of the partner, the duration of
376 the signal was gradually increased along the conditioning²⁵ [trials 1-3: two seconds, trials 4-5:
377 five seconds, trials 6-7: 10 seconds, trials 8-9: 15 seconds, trials 10-12: 20 seconds]. To allow
378 the recording of the vocalisations produced during the anticipatory phase, only the visual
379 stimulus was prolonged and not the auditory stimulus which was kept at two seconds for all
380 trials. Only trials containing a signal (and thus an anticipatory phase) of 20 seconds were
381 analysed. In order to test for anticipation, we needed to disrupt the associative learning. We
382 thus delayed the entrance of the supposedly expected partner on the last and twelfth trial: the
383 signal stopped but the partner entered only after one and a half minute (divided into three 30s-
384 phases, named phases 1, 2 and 3).

385 BEHAVIOURAL MEASURES

386 Behaviours were monitored using a camera (Bosh, Box 960H-CDD) and annotated using *The*
387 *Observer*© *XT 14.0* (Noldus, The Netherlands) software. The squared room was split in 16
388 equally dimensioned zones to assess the mobility and exploratory behaviour of the piglet: every
389 time the shoulders of the piglet crossed a zone, a zone change was scored. The following
390 behaviours were monitored and standardised per minute for each phase: time spent near
391 conspecifics' and human' door zones, time spent watching conspecifics' and human' doors,
392 number of time the piglet watched the conspecifics' and human' doors, number of zones
393 explored, average time spent per zone, time spent static in the room. Behavioural scores were
394 then calculated to quantify global responses (see below).

Manuscript – Submission 1 – piglets anticipation grunts

395 ACOUSTIC MEASURES AND ANALYSES

396 Acoustic monitoring

397 Vocalisations were recorded with a AKG C314 microphone placed in the center of the room
398 and at one-meter-high, connected to a Marantz MD661MK2 recorder. Vocalisations produced
399 during each phase of the trial were manually annotated per vocal type (grunt, squeak, bark,
400 scream and mixed calls), after visual inspection of spectrograms on Praat® software. Only
401 grunts were deeply analysed as they were the most expressed. However, additional
402 observational data on other call types are available in the supplementary document (fig. S1).

403 Acoustic measures on grunts

404 A spectro-temporal analysis was performed with custom-written codes using the Seewave R
405 package²⁶ implemented in R²⁷. After a 0.2-8 kHz bandpass filtering ('fir' function), a
406 standardised grunt was detected when amplitude crossed a 5% amplitude threshold ('timer'
407 function) to measure the duration. After amplitude normalisation, the following spectral
408 parameters were calculated ('specprop' function, FFT with Hamming window, window length
409 = 512, overlap = 50%): mean, median, first (Q25) and third (Q75) quartiles, interquartile range
410 (IQR), centroid and mode (all in Hz). The grunt dominant frequency (in kHz) was also
411 calculated ('dfreq', 50% overlapping FFTs, window length = 512), which is the mean over the
412 grunt duration of the frequencies of the highest level of energy. Parameters measuring noisiness
413 and entropy of the grunt were: Shannon entropy (sh), Spectral Flatness (Wiener entropy, sfm)
414 and Entropy (H) [combining both Shannon and Temporal envelop entropy, length = 512,
415 Hilbert envelop). Two linear acoustic parameters were used: the logarithm of grunt duration
416 and a built-in spectral acoustic score with all spectral parameters (see below). Table of acoustic
417 data available in supplementary material (table S4).

418 STATISTICAL ANALYSES

Manuscript – Submission 1 – piglets anticipation grunts

419 *Behavioural and acoustic scores*

420 To assess changes in global behavioural/acoustic responses during anticipation, parameters
421 were used to build scores using multivariate analyses carried out in two steps. First, a linear
422 discriminant analysis was computed on a subset of data containing the first two phases of the
423 test, maximizing differences between groups of an *ad hoc* factor ‘phase:treatment:partner’. Two
424 behavioural scores (LD1 and LD2) and one spectral acoustic score (LD1) were built. On the
425 remaining dataset (trial 12: phases 1, 2, 3 for which the entrance of the partner was delayed), a
426 projection was computed on LDs scores, allowing to test for differences in behavioural/acoustic
427 space(s).

428

429 *Statistical tests and validation*

430 We tested for differences in LDs scores since the question using a delayed entrance of the
431 partner was to know whether the piglets would keep the state, they would have during
432 anticipation, return to the state they had in initial phase or exhibit intermediate response. All
433 statistics were carried out on R²⁷. A linear mixed effect model (‘lmer’ function, ‘lme4’ R
434 package) was built to test two-way interactions between factors ‘phase of the trial’ (phases: -1,
435 0, 1, 2, 3), ‘partner’ (Human or Conspecifics) and ‘treatment’ (additional H+ or minimal human
436 contacts H). The factor ‘replicate’ (first or second) was also tested in interaction with
437 ‘treatment’ and ‘partner’. Piglet’s identity was put as random factor (repeated measures per
438 piglet). This model was used to test for behavioural scores (LD1 and LD2), the spectral acoustic
439 score (LD1) and the duration score (log). For vocal rhythm (inter grunt interval), the model was
440 simplified to the study of the anticipation phase only (phase 0), since the metric calculated
441 highly depended on the number of observations. The following two-way interactions were
442 tested: ‘partner’ and ‘treatment’, ‘replicate’ and ‘partner’, ‘replicate’ and ‘treatment’. To test
443 for biases in the piglet’s location in the room prior to the emission of any signal (phase -1), the

Manuscript – Submission 1 – piglets anticipation grunts

444 time spent near to the upcoming partner's door (parameter loading the most on the LDs), was
445 used as response variable and trials were grouped in a three-level factor: 'beginning: trials 2-
446 4', 'middle: trials 6-8' and 'end: trials 10-12'. The model tested the three-way interaction
447 between 'trial', 'partner' and 'treatment' and two-way interactions between 'replicate' and
448 'partner' or 'treatment'. All linear models were validated by visual inspection of the
449 symmetrical and normal distribution of the residuals ('plotresid' in 'RVAideMemoire' R
450 package). Anovas were computed on models to test for significant effects of explanatory
451 variables ('car' R package). Model estimates and pairwise post hoc tests were computed using
452 Tukey correction for multiple testing ('lsmeans' R package). A complete report of statistics is
453 available as supplementary material (tables S1-S3).

454 **Discussion**

455 The paradigm developed in this study was built to analyse in piglets the acoustic expression of
456 anticipation of (pseudo)-social events: arrival of two pen mates or a familiar human. We tested
457 two degrees of familiarity with a human, created by handling sessions given to half of the piglets
458 proven to induce a positive link with the human.

459 The behavioural analysis showed that piglets were able to anticipate the social reunion: piglets
460 did show a short-term specific response during the anticipation phase compared to the other
461 phases (approach of zone where partners entered during the signal, attention behaviours toward
462 this location, and specific vocal expression, at least for one of the possible partners). When the
463 arrival of the partner was delayed, the duration of grunts increased for both partner. Longer
464 grunts had already been associated to negative emotional valence^{18,19,28}, which confirms that
465 the delay lead in piglets to a negative emotional state, a situation of non-correspondence with
466 expectation, whatever the partner. These results allow us to conclude that we did succeed in
467 generating a specific anticipatory state during the tests. Those changes were not solely due to

Manuscript – Submission 1 – piglets anticipation grunts

468 the signal emission, as some were persistent even after the signal (e.g. spatial position). We thus
469 confirm the cognitive ability of weaned piglets for associative learning, and for developing
470 expectations from their environment¹².

471 Piglets expressed different behaviours toward partners, showing a preference for their
472 conspecifics compared to the familiar human, which reflect different emotional values (valence,
473 intensity) of the partners. Piglets spent more time near the area of the room where the
474 conspecifics were supposed to enter along the condition sessions, compared to the area where
475 the human partner was supposed to enter. In addition, during the delay phase, piglets expecting
476 conspecifics expressed reactions similar to the anticipation phase, whereas piglets expecting a
477 human rather show reactions similar to the period before the anticipation (isolation). In addition
478 to confirm the ability in piglets to anticipate social reward, those behavioural data confirm the
479 preference in isolated piglets for their conspecifics that represent a stronger positive valence
480 than the arrival of a familiar human. Vocal expression differed between partners and were in
481 line with behavioural observations. The inter-grunt interval was lower when piglets were
482 expecting conspecifics. Morton's rules explain that the rhythm of a behaviour can be positively
483 linked to motivation²⁹. Thus an increase in vocal activity when expecting conspecifics may be
484 explained by the expression of a higher motivation toward this reward compared to the human
485 reward, and thus a higher arousal.

486 This allowed us to measure the vocal expression of anticipation according to the event that is
487 anticipated: arrival of pen mates or a familiar human (high familiarity after positive interactions
488 versus lower familiarity). Considering the spectro-temporal features of grunts, although we
489 failed in measuring a change in grunt duration during the anticipation of pen mates, we found
490 an increase in duration after the signal stopped, when the entrance of pen mates was delayed.
491 This latter phase is certainly a context with a negative valence (social isolation) and piglets may
492 express the negative valence of this context in the duration of their grunt. In fact, several

Manuscript – Submission 1 – piglets anticipation grunts

493 examples of the literature show that grunt duration is higher in negative contexts^{18,28}. The
494 duration of piglets' grunt was not changed during anticipation. Two non-exclusive hypotheses
495 can be raised to explain this result: 1) piglets learned that something positive was going to
496 happen when entering the room (either the arrival of its penmates or the arrival of a human with
497 positive contacts, but not an isolation) and expressed a non-specific positive state during the
498 initial phase, so grunt produced during the initial phase are already 'positive grunt', 2)
499 anatomical constraints of piglets' vocal tract does not allow to shorten the grunts.

500 In a recent study, Briefer *et al.*¹⁹ showed that vocalisations (but not specifically grunts although
501 they are usually over represented in datasets) recorded in positive contexts lasted $0.34 < 0.42 <$
502 0.51 seconds. Considering all grunts produced during the initial phase in our study, the grunt
503 duration is on average of $0.13 < 0.27 < 0.41$ seconds (table S4). This may be in line with the
504 first hypothesis, although the second hypothesis cannot be ruled out. To disentangle these
505 hypotheses, we would need to measure grunt duration in a two-way associative learning with a
506 positive and a negative social context (isolation vs. arrival of conspecifics), however the aim of
507 the present study was to compare various positive contexts.

508 Piglets expecting a human already produced longer grunts during the anticipation phase, grunt
509 that remained longer after the signal stopped, when the entrance of the human was delayed
510 (similarly to when the entrance of penmates was delayed). An increase in grunt duration during
511 the anticipation phase may mean that having a human as reward, instead of conspecifics may
512 be perceived negatively by piglets. We found an average duration for the phase of $(0.031 < 0.35$
513 < 0.39 , table S4), value that are similar to what Briefer *et al.*¹⁹ found for negative contexts. This
514 result is surprising because our behavioural data showed that additional contacts had a positive
515 effect on human-piglet relationship: piglets remained closer to the human (fig. S1). So for at
516 least half of the piglets we can conclude that the presence of the human was positive compared
517 to being isolated before the conditioning took place. In addition, the treatment had no effect on

Manuscript – Submission 1 – piglets anticipation grunts

518 grunt duration comparing the initial and anticipation phases. So we can conclude a smoothing
519 of the differences in familiarity degrees along the conditioning. Since anticipation extrapolate
520 emotional states³⁰ and that during the conditioning, two different output may arise in one trial
521 (either human or conspecifics), we can hypothesize, that piglets may experience a cognitive
522 bias. Piglets may rank the two possible outputs, increase the positivity of having conspecifics
523 and increase the negativity of having a human arriving. In that case, vocal expression of
524 anticipation of a human may be already the expression of a frustration rather than a positive
525 emotion of lower intensity compared to anticipating conspecifics.

526 Spectral features of grunt changed drastically regarding the quality of the partner and contrary
527 to grunt duration, changes were specific from the anticipation phase. Indeed, the spectral score
528 increased when anticipating pen mates and decreased when anticipating the human but it
529 returned to similar values as during the initial phase after the signal stopped, when the entrance
530 of the partner was delayed. Therefore, the acoustic spectral score did not vary the same way
531 depending on the quality of the partner: when expecting conspecifics, piglets produced grunt
532 with higher spectral noise whereas they produced grunts with a higher frequency range and
533 temporal noise when expecting a human. We can hypothesize that these rapid spectral changes
534 are linked to rapid changes in the emotional arousal of piglets. Indeed harmonicity decreases
535 with arousal in grunts¹⁷. Another measure would confirm such a hypothesis: for example heart-
536 rate and its variability would be good indicators the arousal of the animals during anticipation
537 phase and not for valence¹².

538

539 To conclude, we showed that piglets were able to express behavioural and vocal flexibility
540 when anticipating (pseudo)social events. In addition, grunts were spectrally and temporally
541 different whether they were expecting a social reunion or an arrival of a familiar human. More
542 interestingly, we also showed that analysing spectro-temporal properties of grunts allowed

Manuscript – Submission 1 – piglets anticipation grunts

543 distinguishing between contexts (violation of expectations, positive human handling). Thus,
544 acoustic analyses and especially of grunts, that are the most expressed type of vocalisations in
545 pigs, allow tracking subtle changes in emotional states that behavioural analyses could not. Our
546 original results in vocal features claim the possibly to better explore emotional states in non-
547 verbal animals than a mere behavioural investigation.

548

549 **Authors contributions**

550 Conceived and designed the experiment (A.V., C.T., A.B., C.G.). Performed the experiment
551 (A.V., C.G., A.H., M.D.). Collection and edition of the acoustic and behavioural data (A.V.,
552 C.G., A.H., M.D.). Statistical analyses (A.V.). Contributed to the writing of the manuscript
553 (A.V., C.T., A.B.).

554 **Acknowledgments**

555 We acknowledge all the technical staff at UEPR: especially Patrick Touanel and Marie-Hélène
556 Lohat, who largely participated in handling the piglets. We thank Eric Siroux who helped
557 building the acoustic chamber at the beginning of the experiment and Remi Resmond for great
558 discussion about statistics. This project is part of the SoundWel project in the framework of the
559 Anihwa Eranet and funded by ANR 30001199.

560

561 **Data availability**

562 The datasets used and/or analyzed during the current study are available from the corresponding
563 author on reasonable request.

564

565 **References**

- 566 1 Spruijt, B. M., van den Bos, R. & Pijlman, F. T. A concept of welfare based on reward
567 evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state
568 of reward systems. *App. Anim. Behav. Sci.* **72**, 145-171 (2001).
- 569 2 Mendl, M., Burman, O. H. P. & Paul, E. S. An integrative and functional framework for
570 the study of animal emotion and mood. *Proc. R. Soc. Lond. [Biol.]* **277**, 2895-2904,
571 doi:10.1098/rspb.2010.0303 (2010).
- 572 3 Watters, J. V. Searching for behavioral indicators of welfare in zoos: uncovering
573 anticipatory behavior. *Zoo Biol.* **33**, 251-256, doi:10.1002/zoo.21144 (2014).
- 574 4 Boissy, A. *et al.* Assessment of positive emotions in animals to improve their welfare.
575 *Physiol. Behav.* **92**, 375-397 (2007).
- 576 5 Van den Berg, C. L. *et al.* Isolation changes the incentive value of sucrose and social
577 behaviour in juvenile and adult rats. *Behav. Brain Res.* **106**, 133-142,
578 doi:[https://doi.org/10.1016/S0166-4328\(99\)00099-6](https://doi.org/10.1016/S0166-4328(99)00099-6) (1999).
- 579 6 McGrath, N., Burman, O., Dwyer, C. & Phillips, C. J. C. Does the anticipatory
580 behaviour of chickens communicate reward quality? *App. Anim. Behav. Sci.* **184**, 80-
581 90, doi:10.1016/j.applanim.2016.08.010 (2016).
- 582 7 Anderson, C., Yngvesson, J., Boissy, A., Uvnäs-Moberg, K. & Lidfors, L. Behavioural
583 expression of positive anticipation for food or opportunity to play in lambs. *Behav.*
584 *Process.* **113**, 152-158, doi:<https://doi.org/10.1016/j.beproc.2015.02.003> (2015).
- 585 8 Clegg, I. L. K. & Delfour, F. Cognitive judgement bias is associated with frequency of
586 anticipatory behavior in bottlenose dolphins. *Zoo Biol.* **37**, 67-73,
587 doi:10.1002/zoo.21400 (2018).

Manuscript – Submission 1 – piglets anticipation grunts

- 588 9 Imfeld-Mueller, S. & Hillmann, E. Anticipation of a food ball increases short-term
589 activity levels in growing pigs. *App. Anim. Behav. Sci.* **137**, 23-29,
590 doi:<https://doi.org/10.1016/j.applanim.2012.01.012> (2012).
- 591 10 Moe, R. O., Bakken, M., Kittilsen, S., Kingsley-Smith, H. & Spruijt, B. M. A note on
592 reward-related behaviour and emotional expressions in farmed silver foxes (*Vulpes*
593 *vulpes*)—Basis for a novel tool to study animal welfare. *App. Anim. Behav. Sci.* **101**,
594 362-368, doi:<https://doi.org/10.1016/j.applanim.2006.02.004> (2006).
- 595 11 Peters, S. M., Bleijenberg, E. H., van Dierendonck, M. C., van der Harst, J. E. & Spruijt,
596 B. M. Characterization of anticipatory behaviour in domesticated horses (*Equus*
597 *caballus*). *App. Anim. Behav. Sci.* **138**, 60-69,
598 doi:<https://doi.org/10.1016/j.applanim.2012.01.018> (2012).
- 599 12 Imfeld-Mueller, S., Van Wezemaal, L., Stauffacher, M., Gygax, L. & Hillmann, E. Do
600 pigs distinguish between situations of different emotional valences during anticipation?
601 *App. Anim. Behav. Sci.* **131**, 86-93, doi:[10.1016/j.applanim.2011.02.009](https://doi.org/10.1016/j.applanim.2011.02.009) (2011).
- 602 13 van den Bos, R., Meijer, M. K., van Renselaar, J. P., van der Harst, J. E. & Spruijt, B.
603 M. Anticipation is differently expressed in rats (*Rattus norvegicus*) and domestic cats
604 (*Felis silvestris catus*) in the same Pavlovian conditioning paradigm. *Behav. Brain Res.*
605 **141**, 83-89 (2003).
- 606 14 Briefer, E. F. Vocal expression of emotions in mammals: mechanisms of production
607 and evidence. *J. Zool.* **288**, 1-20, doi:[10.1111/j.1469-7998.2012.00920.x](https://doi.org/10.1111/j.1469-7998.2012.00920.x) (2012).
- 608 15 Opiol, H., Pavlovski, I., Michalik, M. & Mistlberger, R. E. Ultrasonic vocalizations in
609 rats anticipating circadian feeding schedules. *Behav. Brain Res.* **284**, 42-50,
610 doi:<https://doi.org/10.1016/j.bbr.2015.02.003> (2015).

Manuscript – Submission 1 – piglets anticipation grunts

- 611 16 Tallet, C. *et al.* Encoding of situations in the vocal repertoire of piglets (*sus scrofa*): a
612 comparison of discrete and graded classifications. *PloS one* **8**, e7184,
613 doi:e7184110.1371/journal.pone.0071841 (2013).
- 614 17 Linhart, P., Ratcliffe, V. F., Reby, D. & Spinka, M. Expression of emotional arousal in
615 two different piglet call types. *Plos One* **10**, doi:10.1371/journal.pone.0135414 (2015).
- 616 18 Friel, M., Kunc, H. P., Griffin, K., Asher, L. & Collins, L. M. Positive and negative
617 contexts predict duration of pig vocalisations. *Sci. Rep.* **9**, doi:10.1038/s41598-019-
618 38514-w (2019).
- 619 19 Briefer, E. *et al.* Vocal expression of emotional valence in pigs across multiple call types
620 and contexts. *PeerJ Preprints* **7**, e27934v27931, doi:10.7287/peerj.preprints.27934v1
621 (2019).
- 622 20 DeBoer, S. P. *et al.* Does the presence of a human affect the preference of enrichment
623 items in young, isolated pigs? *App. Anim. Behav. Sci.* **143**, 96-103,
624 doi:<https://doi.org/10.1016/j.applanim.2012.10.007> (2013).
- 625 21 Tallet, C. *et al.* Behavioural and physiological reactions of piglets to gentle tactile
626 interactions vary according to their previous experience with humans. *Livestock Sci* **167**,
627 331-341, doi:10.1016/j.livsci.2014.06.025 (2014).
- 628 22 Bensoussan, S., Tiget, R., Meunier-Salaün, M.-C. & Tallet, C. Broadcasting human
629 voice to piglets (*Sus scrofa domestica*) modifies their behavioural reaction to human
630 presence in the home pen and in arena tests. *App. Anim. Behav. Sci.*, 104965,
631 doi:10.1016/j.applanim.2020.104965 (2020).
- 632 23 Buttner, K., Czycholl, I., Basler, H. & Krieter, J. Effects of an intensified human-animal
633 interaction on tail biting in pigs during the rearing period. *J. Agric. Sci.* **156**, 1039-1046,
634 doi:10.1017/s002185961800103x (2018).

Manuscript – Submission 1 – piglets anticipation grunts

- 635 24 Clegg, I. L. K., Rodel, H. G., Boivin, X. & Delfour, F. Looking forward to interacting
636 with their caretakers: dolphins' anticipatory behaviour indicates motivation to
637 participate in specific events. *App. Anim. Behav. Sci.* **202**, 85-93,
638 doi:10.1016/j.applanim.2018.01.015 (2018).
- 639 25 Reimert, I., Bolhuis, J. E., Kemp, B. & Rodenburg, T. B. Indicators of positive and
640 negative emotions and emotional contagion in pigs. *Physiol. Behav.* **109**, 42-50,
641 doi:10.1016/j.physbeh.2012.11.002 (2013).
- 642 26 Sueur, J., Aubin, T. & Simonis, C. Seewave, a free modular tool for sound analysis and
643 synthesis. *Bioacoustics* **18**, 213-226, doi:10.1080/09524622.2008.9753600 (2008).
- 644 27 Team, R. C. R: *A language and environment for statistical computing*. . [https://www.R-](https://www.R-project.org/)
645 [project.org/](https://www.R-project.org/) (2017).
- 646 28 Briefer, E. F., Vizier, E., Gygax, L. & Hillmann, E. Expression of emotional valence in
647 pig closed-mouth grunts: Involvement of both source- and filter-related parameters. *J.*
648 *Acoust. Soc. Am.* **145**, 2895-2908, doi:10.1121/1.5100612 (2019).
- 649 29 Morton, E. S. On the occurrence and significance of motivation-structural rules in some
650 bird and mammal sounds. *Am. Naturalist* **111**, 855-869 (1977).
- 651 30 Dudink, S., Simonse, H., Marks, I., de Jonge, F. H. & Spruijt, B. M. Announcing the
652 arrival of enrichment increases play behaviour and reduces weaning-stress-induced
653 behaviours of piglets directly after weaning. *App. Anim. Behav. Sci.* **101**, 86-101 (2006).
- 654
- 655