

## Dear Editor,

Thank you for your review. We reply point by point to your comments below and citing the manuscript. Generally, we did not expect to be asked to include other data or analyses at this point, after already three reviews. There are some misunderstandings that are solved in our responses. We explained again our choices, and the rationale of the present article in our responses. In addition, we would appreciate to receive the feedback of the reviewers to our preceding versions. Did they validate our modifications, that all answered their critics? Thank you for this.

Below, the colour code is as follows: your letter in black and our response in blue.

I thank you for following my recommendations, especially for reorganising the discussion. The discussion is much clearer now. It is still a bit tortuous and not fully convincing. You conclude both that the HAR is more positive in handled piglets and is increased with conditioning [...]

The behavioural results of the standard reunion test (silent and static human) show that, indeed, before conditioning handled piglets show a higher proximity to the human (line 493 "positively handled piglets expressed a higher attraction toward the human than non handled piglets (ReuPC1)"). However, after the conditioning, we found no evidence of a difference in attraction toward the human between experimental groups (line 496 "non handled piglets expressed a similar attraction toward the human as positively handled piglets"). The behavioural results of the conditioning sessions show that both groups increased proximity to the human (line 501 "over time and for both treatments (H and H+), piglets expressed a higher attraction toward the human"). So regarding proximity to the human, the difference between experimental groups is significant only before the conditioning and is attenuated by the conditioning (line 506 "Since no evidence of any interaction between time and treatment was found, no conclusion on differential developmental trajectories between treatments can be drawn, but a parallel development of the human-piglet relationship in both groups, when considering the proximity").

It is true that the handled piglets expressed more investigation of the room than non handled piglets, during (fig. 4A and B) and after the conditioning (fig. 2). We hypothesized that the human-animal relationship may arise sequentially: first increasing proximity, second increasing investigation of the environment in the presence of the human in the discussion. This hypothesis is suggested in the discussion and would require another experiments to test it. See line 526 "We hypothesize a sequential establishment of a positive HAR over time: firstly with a decrease of attentive state and an increase in proximity and accepted contacts, and secondly with a disinterest of human contacts and the expression of natural foraging behaviour. The latter may require a higher exposure time"

[You conclude] and that the short and high-pitched grunts are indicative of a positive emotion. So there should be more of these grunts at the end of conditioning, which does not seem to be the case.

You may be considering the results of the standard test, with the static and silent human. When considering the conditioning sessions, the effect of time during the conditioning did not interact with the treatment but with the location: all piglets (handled at weaning or not) located close to the human showed higher pitched grunts.

If we go into more detail, the standard reunion test (static and silent human) may create some frustration for the piglets that have been used to receive additional positive contacts from the human and do not receive anything during this tests. This may explain why piglets grunts do not change the same way when close to the human during the test (especially the H+ ones). Of course it is a hypothesis. This hypothesis was actually in [V1 of the manuscript](#), see discussion line 497-509 but was removed in v2 and v3 following the review and advice to make the discussion clearer and more straight to the point.

There must be a more elegant interpretation. If I summarise the results:

**Behaviour:** during the tests, the piglets positively handled stay closer to the human. The same is obtained in piglets not positively handled after they receive the conditioning, so that you can conclude that the same level of HAR is obtained after conditioning in all piglets. Also, the piglets positively handled explore more and are more mobile during the tests (both before and after the conditioning).

**Grunts:**

- During isolation phase: longer and lower pitch grunts
- Positively handled piglets have shorter and higher pitched grunts compared to non-positively handled ones, whatever the situation → more related to mood than to the specific context of the test.

Your explanation might be one the hypothesis yes, but we had mentioned the main ones from the knowledge we have on pigs and vocalisations. Your point is discussed lines 546-553 "We can thus hypothesize this may also be reflected in the way piglets vocalise, in general. 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."

- Piglets emit shorter and higher pitched grunts when in proximity to humans, especially when the human is not familiar (e.g. the difference is more marked in non-handled piglets and – in all piglets- more marked before conditioning).

The test you consider here is the standard reunion with a silent and static human (see fig. 3). When considering the conditioning session, during which the piglets received additional positive contacts the grunts are shorter and higher pitched for all piglets when close to the human (fig. 5A and 5C).

The behaviour of piglets should help you to interpret these variations in grunts:

- What difference in behaviour do you see in absence vs presence of the human? Such results are not reported. They could help you better relate grunts and behaviour.

We can see you also commented the manuscript (line 318) "what about behaviour during isolation vs; reunion; This seems important to interpret the results". We do not agree with you, this is not important to interpret the results, as this is not the aim of this article. The purpose of the experiment was to compare the behaviour towards the human in two groups of piglets that have been exposed differently to this human and not to describe behavioural variation between isolation and reunion with a human. Our objective and hypothesis are exposed in the introduction and it has been validated by the reviewers.

This type of analyse has been published many times, and you may refer to our recent publication by Villain et al 2020 (doi: 10.3389/fvets.2020.577433) in which the difference of reaction during isolation and reunion has been analysed, after interactions with a human being or not, as in the present article.

It was an experimental choice to have this isolation phase before: indeed, isolation is known to be perceived as negative by the piglets, and gave us a negative control regarding the vocal expression of piglets. Analysing the behaviour of this phase would not answer to any of our predictions (see introduction line 102 "We predicted that if grunts reflect the quality of the human-pig relationship, then 1. A period of positive handling given by a human should modulate piglets vocal expression in presence of the human, leading to grunts exhibiting markers of positive states (higher pitched and shorter grunts), 2. Spatial proximity toward the human should influence the spectro-temporal structure of grunts (higher pitched and shorter grunts)."). - The strong parallel I see between behaviour and grunts in your study is about exploration: Handled pigs both explore more the test pen and emit shorter and higher pitched grunts. These two seem to go together. It suggests that short and high-pitched grunts have something to do with exploration (and vice-versa). And probably the handled piglets feel more confident (thanks to a positive HAR) and explore more.

It is true that H+ piglets do explore more (see fig. 2 and 4) and this hypothesis of being more confident is already suggested in the discussion, although in a different way. See line 517 "In addition, this could also be interpreted in terms of attachment to the human. Indeed, attachment to a human may facilitate exploration of novel environments or objects, as shown in dogs (Palmer & Custance, 2008). A period of positive handling at weaning may provide an environment secure enough for the piglets to explore their environment in the presence of the human. Attachment has also been hypothesised in the lambs-human relationship (Tallet et al., 2009)."

However, based on the current data, we are not sure we can relate the exploratory behaviours (especially from H+ piglets) to the structure of vocalisations. Grunts were not labelled in the recordings according to what the piglets were doing (investigating floor, for example) but where they were located (away or close to the human). What the data shows is that being close to the human or away from them affects the structure of vocalisations, especially when the human is providing positive contacts. The question of

exploration is a relevant question to ask, however another experimental design would be needed to test it.

- The fact that short and high-pitched grunts are expressed preferably in proximity to human especially when it is not very familiar goes in the same direction: we can think that the human is more explored when not familiar

However, you refer to the standard reunion test and this statement is not true during the session of additional positive contacts of the conditioning, during which piglets from both experimental groups exhibited shorter and higher pitched grunt when close to the human. In other words, the behaviour of the human affects the structure of piglets' grunts. This point is discussed section line 598. "On the one hand, time decreased vocal reactivity to human proximity during a standard reunion with a static human. On the other hand, no evidence of a decrease in vocal reactivity to human proximity was found during sessions of additional positive contacts. This would mean that positive interactions with piglets consistently triggers the production of shorter and higher pitch grunts"

My feeling is that the short and high-pitched grunts are related to "positive" exploration (as opposed to "negative" exploration = looking for escape). You may not agree with such an interpretation. I may have misunderstood your results (I got a bit lost I must admit).

This might be an interpretation and Marchant et al (2001) had already tried this type of interpretation of the meaning of each vocalisation. This would need to be investigated further, as grunts are not solely expressed during exploration.

Also, like Reviewer 2, I am frustrated by the fact that your analyses are essentially on the quality of grunts and not their quantity. I would like you to consider the above reasoning and try to make your interpretations more convincing.

We understand that there might be a frustration of discovering new ways of considering vocalisations. Pigs do vocalise a lot, unlike ruminants, and the quantity of their vocalisation is not directly linked to their emotional state in a linear way. So, with this article, we focus on the quality of the vocalisations. The core of the paper is to ask the question whether we can find reliable indicators of the HAR in the acoustic structure of piglets' call, and this is a choice we endorse. We are not in favour of adding data on the quantity of vocalisations. In addition, and that is a pity to have to say this, we already answered to this point earlier, after the first round of review, and had no feedback on the second round of reviews.

I also have minor comments on the writing. I include them on your manuscript (file attached).

Your comments were taken into account in v4 of the manuscript. See details below:

- the term "minutes" in methods was replaced by "min" as requested as well as space between before the units.

- comment line 144 "briefly summarise the procedure because you refer to it L 164". A sentence was added to briefly describe the procedure of additional positive contacts. See line 143 " Briefly, the behaviour of the human toward the piglet was adapted to the

reaction of each animal and included four steps: (1), the handler hold out the hand towards the animal; (2) if the piglet did not move away, the handler tried to touch it; (3) if the piglet accepted being touched, the handler softly stroked it along the body with the palm of her hand; and (4) once it accepted being stroked, the handler scratched it along the body with her fingers. Scratching consisted in rubbing the skin of the piglets with the finger tips and applying more pressure than stroking. No specific body part of the piglets was more considered than another”

-comment line 165 “The two sentences are redundant”. The sentence was clarified in the method regarding the exposure time to the human by piglets for each step of the experiment. See line 171 “ Therefore, at the beginning of the conditioning, H+ piglets were already familiar with the human and procedure, whereas H piglets were unfamiliar with the human. During the conditioning, the same sessions occurred in both treatment groups (H and H+). After the conditioning, all piglets were familiar with the human, but treatment groups had a different time of exposure to them.”

- comment on acoustic analysis section line 211 “i don't understand the sentence”. This sentence refers to classic controls prior to acoustic analyses and was clarified in the methods. See Line 211 “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 and compared it with the general frequency range of the grunts. To avoid measuring masking effect of the background noise, grunts were filtered using a 0.2-8 kHz bandpass filtering (‘fir’ function). As a consequence, all results presented in this study are on a 0.2-8 kHz frequency range, and no conclusions on frequency components of grunts below this 200 Hz threshold are drawn here.”

- comment line 257 “not introduced before: to be defined”. If you refer to the two experimenters, they are introduced in the “treatment: positive handling at weaning” section. See line 149 “Two experimenters (‘AV’ and ‘AH’) performed these sessions (both women, both between 1.70-1.73 m tall, with a balanced number of pens attributed to each of them). “

- comment line 280 “for all models: explain why all interactions were not tested”. See line 263 “All explanatory variables used in the models and interactions between them were built in respect to the experimental design and to allow biological interpretations. As a consequence, not all interactions between all variables were made. They are fully explained in the subsequent sections.”

- comment on figure 1: “i am not convinced of your interpretation of PC3. It seems to me that it is rather look at human as opposed to exploring the pen”. If you look at table 2, for PC3, the absolute highest loading is “Time spent investigating floor” with -42.2. “time watching human” is 25.5 on PC3 whereas it is -52.9 on PC1. “Nb looks toward H” is -31.6 on PC3 whereas it is -43.7 on PC2. So, we had to disagree, PC3 is negatively correlated with “time spent investigating floor” because the other behaviour 1) do not load enough on PC3 and 2) load better other PCs and thus explain more other PCs than PC3.

Céline Tallet, on behalf of the authors