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► **To cite this version:**

Céline Tallet, Kardiatou Sy, Armelle Prunier, Raymond Nowak, Alain Boissy, et al.. Behavioural and physiological reactions of piglets to gentle tactile interactions vary according to their previous experience with humans. *Livestock Science*, 2014, 167, pp.331-341. 10.1016/j.livsci.2014.06.025 . hal-01129853

**HAL Id: hal-01129853**

**<https://hal.science/hal-01129853v1>**

Submitted on 27 May 2020

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2 to their previous experience with humans

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## 22 Abstract

23 Providing piglets with repeated stroking and brushing leads to behaviours of affinity towards  
24 their handler, but there is still no evidence of physiological modifications. In addition, other  
25 tactile stimulations like scratching have not yet been studied while there are used by pig-  
26 keepers. In Thus, the present experiment aimed at determining the consequences of stroking  
27 and scratching weaned piglets on their later behavioural, cortisol and cardiac responses to  
28 human presence and gentle tactile interactions. Four groups of four piglets were weaned at  
29 28 days of age (Day 0) and handled twice a day for 10 min, five days a week, from Day 1 to  
30 28. Handling consisted in standing for 30 s, sitting for 1 min and then stroking and scratching  
31 each piglet for 2 min. Four groups of four piglets were used as controls and received only the  
32 minimal contacts for routine husbandry practices. Behavioural reactions to the presence of  
33 the handler in the home pen (Day 25) and to her presence and departure were observed in  
34 an arena test (Days 26-27). Behavioural and cardiac responses to the handler's presence  
35 and contact were compared in a test pen (Days 33-35). Salivary cortisol levels were  
36 measured in another test pen after 15-min of either contacts with the handler or isolation  
37 (Days 40-43). In the home pen, handled piglets investigated sooner the handler ( $P < 0.001$ )  
38 and spent more time investigating her than control piglets ( $P < 0.05$ ). In the arena test,  
39 handled piglets investigated sooner the handler ( $P < 0.01$ ) and spent more time close to the  
40 handler than control ones ( $P < 0.01$ ). Heart rate of the piglets during stroking and scratching  
41 did not differ between treatments but the root mean square of successive differences in heart  
42 beat intervals was lower in handled than in control piglets when the rear part of the body was  
43 scratched ( $P < 0.01$ ). There was no effect of the previous experience with the handler on  
44 salivary cortisol levels ( $P > 0.05$ ). Overall this study shows that scratching and stroking  
45 piglets during three weeks after weaning changed the way that they interacted with the  
46 handler, being more attracted than control piglets. However, there was no clear difference in  
47 cardiac reactions to scratching and stroking between handled and control piglets, suggesting  
48 that these contacts are perceived positively, regardless of the previous experience. The body  
49 region stimulated may be of importance but it needs further investigation.

50

51 Keywords: pigs, human-animal relationship, behaviour, cardiac activity, cortisol

52

## 53 1. Introduction

54 Many studies showed that a positive human-animal relationship may develop in farm animals  
55 like pigs, cattle, sheep, horses or poultry (Waiblinger, 2009; Hemsworth and Coleman, 2011).  
56 A positive relationship is notably characterised by an absence of fear reactions to humans  
57 and an easiness to handle the animals (Waiblinger et al., 2006). It is favourable to animal  
58 welfare and also enhances performances (Boivin et al., 2003; Hemsworth, 2008) and may be  
59 a source of positive emotions for farm animals (e.g. Tallet et al., 2005; Schmied et al., 2008).  
60 Among the interactions with humans that can be perceived as positive, food provision is an  
61 efficient way of attracting animals (i.e. Hemsworth et al., 1996b; Jago et al., 1999; Sankey et  
62 al., 2010). Gentle tactile interactions have also been tested as a potential way to decrease  
63 fear of humans and to increase attraction to them. In farm animals, this has mainly been  
64 investigated in cattle, sheep and horses. Although some studies do not show a beneficial  
65 effect of gentle tactile stimulations on perception of humans (Boivin et al., 1998; Jago et al.,  
66 1999; Hausberger et al., 2008) most of them indicate that such stimulations are a source of  
67 positive reactions towards humans and induce a decrease of behavioural reactions of fear in  
68 human presence (e.g. Becker and Lobato, 1997; Tallet et al., 2005; Tallet et al., 2011a; Tallet  
69 et al., 2011b; Probst et al., 2012). Very few data are still available in farm animals regarding  
70 the consequences of gentle tactile stimulations on physiological indicators of stress like  
71 cortisol level and heart rate. In beef cattle, gentle touching in early age seems to dampen the  
72 cortisol release at slaughter (Probst et al., 2012) while the reduction is not always significant  
73 when applied only few weeks before slaughtering (Mattiello et al., 2010). Likewise, gentle  
74 stroking has been shown reduce heart rate increase during a veterinary procedure (Schmied  
75 et al., 2010) or in presence of the handler in an arena test (Raussi et al., 2003; Tallet et al.,  
76 2006) in cattle and sheep. To our knowledge, the influence of gentle contact on the  
77 physiological indicators of stress has been poorly investigated in pigs. The cortisol release of  
78 pigs after human exposure was analysed only in two studies (Hemsworth et al., 1986a;  
79 Paterson and Pearce, 1992) and both of them did not show clear effects but the numbers of  
80 pigs studied were very low (four or seven pigs in each group).

81

82 In the literature on farm species, different types of tactile contact were applied to the animals  
83 like stroking (i.e. Tallet et al., 2005 in sheep), brushing (i.e. Tanida et al., 1994 in pigs),  
84 simply touching (i.e. Breuer et al., 2003 in cattle) or holding the animals (Tallet et al., 2009 in  
85 sheep) but they were rarely compared. All these types of interactions may be given by  
86 stockpeople, either at distinct moments or during a (usually short-lasting) sequence of  
87 interactions. Besides the quality of the interactions, the body region being touched is  
88 important for the animals as shown in cattle (Schmied et al., 2008) and rats (Grandin, 2010).  
89 In the study of Schmied et al (2008), cows expressed more positive reactions (e.g. neck  
90 stretching) to stroking at the withers and neck ventral regions than at the lateral chest. This  
91 could be related to the intra-specific social behaviour since intra-specific physical interactions  
92 are directed to preferential parts of the body in cattle for instance (in cattle: Val-Laillet et al.,  
93 2009; Laister et al., 2011) and the human stimulation of the preferred area are more effective  
94 on relaxation postures and physiological indicators compared with other regions (Schmied et  
95 al, 2008).

96

97 The importance of the type of gentle tactile stimulations has been less investigated in pigs  
98 than in cattle or sheep. Yet pig-keepers also interact physically with their animals and pigs  
99 are social animals which may interact physically with their keeper as well p (Hemsworth,  
100 2008). At the intra-specific level, tactile contact (nosing, nibbling, huddling) are important and  
101 pigs are used to rest in close body contact (Hafez, 1975). Pigs perform soft contact with their  
102 nose (Camerlink and Turner, 2013) or more dynamic contact looking like udder massages  
103 around nursing (Torrey and Widowski, 2006). Thus an influence of tactile human contact on  
104 the subsequent reactions of pigs to humans is expected. In pig production, human gentle  
105 interactions may take the form of stroking, touching but also scratching (personal  
106 observations). Stroking could mimic nosing while scratching that involves greater pressure  
107 than nosing would mimic massage. Pigs that are stroked whenever they approach a human  
108 during training sessions express subsequent contact seeking (Hemsworth et al., 1986a;

109 Paterson and Pearce, 1992). To our knowledge, the influence of scratching has not been  
110 studied, while it is used by pig keepers and may have different consequences from stroking.  
111 Tanida et al (1994) used brushing which is close to scratching but differs from it because of  
112 the presence of an object between the animal and the human. They observed that, after two  
113 weeks of treatment, pigs spent more time in contact with humans when they had been  
114 brushed than when they had been stroked. However, in this study, brushing lasted 15 min  
115 per week whereas the duration of stroking depended on the willingness of the pig to  
116 approach the human; the amount of stimulation could have thus influenced the behavioural  
117 response of pigs. In all reported experiments, the body region exposed to the stimulation was  
118 never specified suggesting that there was no target region, but that interactions were  
119 probably provided all along the pigs' back. To our knowledge, no favourite body region was  
120 reported for pigs' social interactions but the rear part of the body could be more often  
121 concerned than the head (personal observation). The head and neck are rather preferential  
122 locations for aggressive interactions (Turner et al., 2006).

123

124 The present experiment aimed at determining the consequences of stroking and scratching  
125 weaned piglets on their later behavioural, cortisol and cardiac responses to human presence  
126 and to tactile interactions. The following hypotheses were tested: 1/ repeated gentle contact  
127 including stroking and scratching leads to a positive relationship with human, 2/ the reactions  
128 to different tactile contact depend on the previous experience with these stimulations.

129

## 130 2. Material and methods

131 The design of the experiment was approved by the local ethic committee (Comité Rennais  
132 d'Ethique en matière d'Expérimentation Animale, number R-2010-CT-02).

133

### 134 2.1. Animals and rearing conditions

135 We studied 32 female piglets (*Sus scrofa domesticus*) allocated to two treatments after  
136 weaning. They were born at the experimental unit of Saint-Gilles (INRA, France) from 11

137 Large White x Landrace sows inseminated by Pietrain semen. Pigs were weaned at  $28 \pm 2$   
138 days of age (Day 0) and transferred to a new building where they were housed in groups of  
139 four in a single room. Weaning weight was similar in all groups varying from  $7.3 \pm 0.7$  kg to  
140  $7.6 \pm 0.6$  kg (mean  $\pm$  SEM within the groups). Siblings were never housed in the same pen,  
141 and piglets from one litter were equally allocated to both treatments. The pens measured 1.2  
142 m x 1.3 m and consisted of slatted floor. The temperature was automatically set at 26°C. The  
143 animals were fed with a standard weaning diet and had *ad libitum* access to food and water.  
144 Food was provided from outside the pen by the stockperson, twice a day. He took advantage  
145 of this presence in the room to visually check the health status of the animals. No health  
146 troubles were observed during the experiment, and so there was no other specific  
147 intervention of the stockperson.

148

## 149 2.2. Treatments

150 *Minimal human contact, H0:* Control animals from four rearing pens received the minimal  
151 amount of daily contact with a stockperson (a 1.70 m high male) required for feeding,  
152 cleaning and health checking. The stockperson wore dark green shirt and pants with brown  
153 shoes .

154 *Additional human contact, H1:* Animals from the four other rearing pens received, in addition  
155 to daily care given by the stockperson as for H0, gentle tactile contact from a handler (a 1.65  
156 m high woman), who wore the same overalls and boots each time she interacted with the  
157 pigs; i.e. blue overalls and dark green boots. This additional contact was given from the day  
158 after weaning and lasted for three and a half weeks (till Day 25, Table 1), with two sessions  
159 per day (except at weekends). Human contact was standardised and consisted in entering  
160 the pen and staying immobile for 30 s, then sitting on a bucket and remaining motionless for  
161 1 min, and finally giving gentle tactile contact to each animal for 2 min. The last phase was  
162 adapted to the reaction of each animal and included four steps: 1), the handler hold out the  
163 hand towards the animal; 2) if the piglet did not move away, the handler tried to touch it; 3) if  
164 it accepted being touched, the handler softly stroked it along the body with the palm of her



165 hand; and 4) once it accepted being stroked, the handler scratched it along the body with her  
166 fingers. Scratching consisted in rubbing the skin of the piglets with the finger tips and  
167 applying more pressure than stroking. The handler provided the stimulation at a rhythm of 3  
168 per second on about 20 cm of length. In addition, the handler spoke to the piglet with a soft  
169 voice.

170

### 171 2.3. Measurements

172 *Test 1. Behaviour towards the handler in the rearing pen at the end of the treatment period*  
173 *(Day 25, Table 1).* We compared the behavioural reaction of H1 and H0 piglets to the  
174 presence of the handler in their home pen. The animals were identified with a number on  
175 their back. The handler entered the home pen and stood motionless for 30 s. Then, she sat  
176 in the centre of the pen and remained motionless without interacting with the animals for 2  
177 min. From videos, the interactions with the handler (looking towards and entering in contact  
178 with the handler, details in Table 2) were recorded. The latency and duration of each item as  
179 well as the number of interactions were calculated for the total observation period.

180

181 *Test 2. Behaviour towards the handler presence and then departure in an arena test (Days*  
182 *26-27, Table 1).* We compared the behaviour of H1 and H0 piglets to the handler (her  
183 presence and absence) in a testing room (3m x 3 m). The floor was divided in 16 identical  
184 zones (0.75 m x 0.75 m) by painted lines. The piglets were individually transported to the  
185 testing room by a disguised stockperson using a cart on wheels filled with straw. The  
186 disguised person (male) wore white coveralls, a white mask, white gloves, sun glasses and  
187 was perfumed in order to avoid individual recognition by the piglets. The test consisted in  
188 three phases of 2 min each: 1. The handler sat in the middle of a wall of the testing pen. She  
189 interacted with the piglet if it made contact with her; 2. The handler left the room and the  
190 piglet remained alone for 2 min; 3. The handler came back at the same place for 2 min. The  
191 testing order and the location of the handler (left/right wall of the test pen) were organized to  
192 alternate according to the pen and treatment of the animals. An experimenter who was not

193 visible by the animals counted the vocalisations (Table 2) during the test. Locomotor activity,  
194 behaviours directed to the handler (Table 2) and the time spent very close to the handler  
195 (corresponding to the two zones in which the handler was present) were determined from  
196 video records. Due to one missing video record, 15 observations for the H1 treatment and 16  
197 for H0 treatment were analysed.

198

199 *Test 3. Changes in behavioural and cardiac activities according to the handler's behaviour in*  
200 *a test pen (Days 33-35, Table 1).* The test aimed at comparing the reactions of the piglets to  
201 strokes and scratches. This test was performed in the same room as Test 2, but in a smaller  
202 pen having the shape of a quarter circle. The two perpendicular walls were 1 m long so that  
203 the handler could easily touch the animal, even if it did not come close to her. It also reduced  
204 the piglets' locomotor activity, limiting the influence of the physical activity on the cardiac  
205 activity.

206 Thirty minutes before the test, each animal was fitted with an elasticised belt by a disguised  
207 experimenter (similar as in Test 1) and stayed in its home pen so that it had time for getting  
208 used to the belt before being tested. Then, each piglet was individually transported to the  
209 testing room in the same manner as in Test 1. In the testing room, the telemetric recording  
210 apparatus (Polar Vantage NV, Elektro Oy, Helsinki, Finland) was fixed to the sternum, under  
211 the belt, by two disguised experimenters. The test was divided in six phases of 2 min: 1.  
212 Isolation in the test pen ("alone"); 2. The handler entered and sat on a bucket in the pen  
213 ("Hsat"); 3. The handler stroke the animal all along the body ("strok1"); 4. The handler  
214 scratched the animal on its head ("scratH"); 5. The handler stroke the animal all along the  
215 body again ("strok2"); and 6. The handler scratched the animal on its rear ("scratR"). The  
216 testing order was organised according to the pen and treatment of the animals. An  
217 experimenter counted the vocalisations (Table 2) during the test via The Observer XT 9.0  
218 (laptop). The behaviours directed to the handler and escape attempts were video recorded  
219 (Table 2).

220 Cardiac activity data were transferred from the receiver to a computer equipped with Polar  
221 HR Analysis 5.04. and exported in text files. Each text file was carefully analysed in order to  
222 delete the errors corresponding to abnormal durations between heart beats probably due to  
223 connection problems (Marchant-Forde et al., 2004). Heart rate (bpm = number of beats per  
224 min) and time interval (in ms) between two consecutive beats were calculated. To evaluate  
225 the heart rate variability and hence the vagal tone, the root mean square of successive  
226 differences (RMSSD) in inter-beat duration was calculated (Porges, 1995; von Borell et al.,  
227 2007) (Porges, 1995; von Borell et al., 2007). Mean bpm and RMSSD were calculated on  
228 each 2-min phase of the test as well as on the first and last 10 s of each phase. Due to  
229 technical problems and to a very high physical activity on two animals, data on 2-min phases  
230 were analysed in 11 H1 and 12 H0 piglets. For the 10-s sequences, data from some more  
231 animals could not be analysed (too much missing values) and the sample size is reported in  
232 the results section (Table 3).

233

234 *Test 4. Cortisol responses to isolation and human contact in a test pen (day 40-43, Table 1).*

235 The test aimed at comparing the cortisol level of the piglets after either 15 min of isolation or  
236 15 min of human contact. [The timing was chosen from a previous experiment published with  
237 the same method as us, showing that salivary cortisol peak is expressed 15 minutes after the  
238 stressor started \(Merlot et al., 2011, Ruis et al., 2001\).](#) It was performed in the same room as  
239 Tests 2 and 3 but in a triangular area delimited in a corner of the room (1 m x 1 m x 1.4 m).  
240 In this way, the handler could easily touch the animal, even if it was not willing to come into  
241 contact. The piglets were individually transported to the testing room in the same manner as  
242 in Test 1. Animals were tested in both situations two days apart, at the same time of the day.  
243 Each test lasted 15 min. During the test in human presence, the handler was asked to stroke  
244 and scratch regularly the piglet. The testing order was organised according to the treatment  
245 of the animals and their pen in a cross-over design. The behaviours directed to the handler  
246 were video recorded (Table 2). To obtain saliva for measure of cortisol levels, at the end of  
247 the test, the handler gave the piglet a cotton bud to chew until it was moistened. Cotton buds

248 were immediately centrifuged at 3000 × g for 15 min at 4°C. Saliva samples were stored at  
249 –20°C until assay. Cortisol was assessed using an immuno-luminescence assay (LIA, IBL,  
250 Hamburg, Germany) as described by Merlot et al. (2012).

251

#### 252 2.4. Recording of piglet behaviour

253 Treatment sessions and all the tests were video recorded with a camera (Panasonic PC25-  
254 2230P33) fixed above the pen linked to a computer equipped with a video acquisition card  
255 and Mpeg recorder (Noldus, Netherlands). From the videos, the behaviour was analysed  
256 during each testing phase with The Observer XT9.0 (Noldus, Netherlands). We carried out  
257 continuous behavioral observations using all pigs as focal animals, even during Test 1 (all  
258 the pigs were observed).

259

#### 260 2.5. Statistics

261 The animal was used as the statistical unit for all measures. The general procedure for the  
262 analyses consisted in running repeated ANOVA analysis (MIXED procedure of SAS 9.2, SAS  
263 Institute Inc.). For Tests 2 and 3, the model included the treatment (H0 and H1), phase of the  
264 test (repeated variable, 1.human presence, 1.isolation, 3.human presence), and their  
265 interaction. The place of the handler in Test 2 (left or right) was also included in a preliminary  
266 model, but as it was never significant, it was removed from the final analyses. For Test 4, the  
267 model included the treatment, the testing condition (isolation or human presence) as a  
268 repeated factor and their interaction. The animal identity was used as a random factor for all  
269 models. For all the models, post hoc comparisons after ANOVA were run using Least Square  
270 Differences (LSD). In order to normalise the distribution of the residual of the models and  
271 meet criteria for parametric statistics, some data (Test 3: RMSSD; Test 4: cortisol level) were  
272 transformed by log(n + 1) (Martin and Bateson, 1994). When the criteria for parametric  
273 statistics could not be met (Test 1, Test 3: behaviour, cardiac activity parameters 10 s before  
274 and after the start of each phase of the test, Test 4: behavioural data), non-parametric tests  
275 (Mann-Withney for the treatment effect, Friedman time effect) were performed (Statview, 5.0,

276 SAS Institute Inc.). Values are presented as means  $\pm$  standard errors of means of non-  
277 transformed data (after ANOVA) or as medians and interquartiles (IQ, after Mann-Whitney  
278 test).  
279 Spearman correlations were calculated to determine potential links between behaviour and  
280 cardiac activity during Test 3 (SAS 9.2, SAS Institute Inc.). The rare behaviours (i.e. climbing  
281 on the human) were analysed by comparing the number of animals by treatment performing  
282 the behaviour with a modified version of the Chi2 test better suited to small samples: the  $2\hat{I}$   
283 test (Arbonnier, 1966).  
284 Results were considered as significant when the probability of the null hypothesis was less  
285 than or equal to 0.05.

286

### 287 3. Results

288 During the handling sessions, all the H1 piglets accepted being gently touched from session  
289 6 onwards and approached the handler when she entered the pen from session 13 onwards.

290

#### 291 3.1. Test 1. Behaviour towards the handler in the home pen at the end of the handling 292 sessions

293 While the handler was standing (30 s), 11 H1 piglets investigated her but only one H0 piglet  
294 did so ( $2\hat{I} = 15.0$ ,  $P < 0.001$ ). Instead, the H0 piglets looked at her more often ( $N = 10$ , versus  
295  $N = 3$  H1 piglets,  $2\hat{I} = 6.62$ ,  $P < 0.05$ ). While the handler was sitting for 2 min, H1 piglets  
296 investigated her sooner (H1: 2.7 s (1.2-4.7 s); H0: 78.9 s (29.1-114.1 s);  $U = 33$ ,  $P < 0.001$ )  
297 and for a longer duration than H0 piglets (H1: 74.4 s (50-97 s); H0: 1.5 s (0-34.3 s);  $U = 27$ ,  $P$   
298  $< 0.001$ ). Six H1 piglets were observed climbing on her but no H0 piglets ( $2\hat{I} = 9.71$ ,  $P <$   
299  $0.01$ ). More H1 than H0 piglets were observed being in contact with her (H1:  $N = 16$ , H0:  $N =$   
300  $9$ ,  $2\hat{I} = 11.69$ ,  $P < 0.001$ ), but less H1 piglets looked at her (H1:  $N = 4$ , H0:  $N = 12$ ,  $2\hat{I} = 8.37$ ,  
301  $P < 0.01$ ). Only H0 piglets expressed avoidance reactions ( $N = 15$ ).

302

#### 303 3.2. Test 2. Behaviour towards the handler presence and departure in the arena test.

304 H1 piglets approached the handler sooner (H1:  $86 \pm 6$  s; H0:  $111 \pm 6$  s;  $F_{1,30} = 9.14$ ,  
305  $P = 0.005$ ) and stood for a longer time near her ( $F_{1,30} = 12.7$ ,  $P = 0.001$ , Figure 1) than H0  
306 piglets. H1 piglets investigated the handler sooner than H0 (H1:  $81 \pm 7$  s; H0:  $103 \pm 7$  s;  
307  $F_{1,30} = 4.67$ ,  $P = 0.04$ ) but for a similar duration (H1:  $4.2 \pm 1.7$  s; H0:  $4.8 \pm 1.6$  s;  $F_{1,30} =$   
308  $0.05$ ,  $P = 0.82$ ). Independently of the treatment, the animals approached and contacted the  
309 handler sooner, and for a longer duration (see Figure 1 for the time spent near the handler)  
310 in phase 3 compared with phase 1 ( $P < 0.05$ ). There was no interaction between treatment  
311 and phase of the test ( $P > 0.05$ ) for the preceding variables. We found a significant phase x  
312 treatment interaction for the time spent looking at the handler ( $F_{1,28} = 7.27$ ,  $P = 0.01$ ): it  
313 increased between phase 1 and 3, and H0 piglets spent more time looking at the handler  
314 than H1 piglets during phase 3 (Figure 1). There was no significant effect of treatment ( $F_{1,30}$   
315  $< 0.71$ ,  $P > 0.05$ ) or phase ( $F_{2,57} < 1.16$ ,  $P > 0.05$ ) and no interaction ( $F_{2,57} < 2.61$ ,  $P >$   
316  $0.05$ ) for the number of zones crossed ( $18 \pm 2$  zones) and the number of low-pitched  
317 vocalisations ( $18 \pm 2$  times). No high-pitched vocalisations were emitted.

318

319 *3.3. Test 3. Changes in behavioural and cardiac activities according to the handler's*  
320 *behaviour in the test pen.*

321 *Behaviour*

322 The number of vocalisations, the time spent investigating the handler and the time spent  
323 without contact with the handler evolved significantly along the phases (Friedman test,  $P <$   
324  $0.04$ , see Figure 2). At the beginning of the test (Hsat phase), H1 piglets expressed more  
325 low-pitched vocalisations than H0 piglets ( $U = 71$ ,  $P = 0.03$ ). In addition, more H1 than H0  
326 piglets expressed high-pitched vocalisations (H1:  $N = 9$ , H0:  $N = 2$ ;  $2\hat{I} = 7.20$ ,  $ddl = 1$ ;  $P <$   
327  $0.01$ ). H1 piglets investigated sooner the handler (H1: 2.0 s (IQ = 5.8 s)) than H0 piglets (H0:  
328 16.8 s (IQ = 46.6 s);  $U = 40$ ,  $P < 0.001$ ) and more H1 piglets climbed on the handler (H1:  $N =$   
329  $8$ , H0:  $N = 2$ ,  $ddl = 1$ ;  $2\hat{I} = 5.31$ ,  $P < 0.05$ ). There was no significant treatment effect on the  
330 time spent investigating the handler ( $P > 0.05$ , Figure 2b). During the second and third  
331 phases of the test (Strok1 and Scrath), H0 piglets spent less time without contact with the

332 handler, ( $U < 61$ ,  $P < 0.01$ ). During the subsequent phases, here was no significant  
333 difference ( $P > 0.1$ , Figure 2c).

#### 334 *Cardiac activity*

335 There was no interaction between treatment and phase ( $F_{5,87} = 1.42$ ,  $P = 0.23$ ) and no  
336 effect of the treatment on the heart rate ( $F_{1,22} = 0.12$ ,  $P = 0.73$ ) but the phase effect was  
337 significant ( $F_{5,87} = 13.49$ ,  $P < 0.0001$ ) (Figure 3a): heart rate decreased significantly  
338 between “Scrath” and “Strok2” phases. The interaction between treatment and phase was  
339 significant for RMSSD (log,  $F_{5,87} = 3.42$ ,  $P = 0.007$ ), i.e. there was no difference at the  
340 beginning but the evolution of the mean RMSSD differed between the treatments (Figure 3b).  
341 For H0 piglets, the mean RMSSD decreased until the “Scrath” phase and then increased  
342 until the end of the test to reach the same level as in the beginning of the test. For H1 piglets,  
343 the mean RMSSD decreased to the end of the test. During the last phase (“ScratR”), the  
344 RMSSD was significantly higher for H0 than for H1 piglets (Figure 3b).  
345 Comparing the last ten seconds of a phase with the first ten seconds of the following phase  
346 (immediate response) demonstrated differences between treatments (Table 3). The heart  
347 rate increased from the “alone” to the “Hsat” phase for H0 and H1 animals. It decreased for  
348 H1 animals between “scrath” and “strok2” and between “strok2” and “scratR”, but did not  
349 evolve for H0. There was no significant difference of RMSSD between last ten seconds of a  
350 phase and first ten seconds of the following phase ( $P > 0.05$ ).

#### 351 *Correlations between behaviour and cardiac activity*

352 The heart rate was negatively correlated with the time spent without contact with the handler  
353 during the “Hsat” phase for piglets from the H0 treatment ( $R_S = -0.73$ ,  $P = 0.007$ ). The  
354 RMSSD was negatively correlated with the time spent lying down without contact to the  
355 handler for H0 ( $R_S = -0.80$ ,  $P = 0.006$ ) and H1 ( $R_S = -0.84$ ,  $P = 0.005$ ) piglets during the  
356 “Strok2” phase. In addition, for H1 piglets, the RMSSD was positively correlated with the time  
357 spent investigating the handler ( $R_S = 0.69$ ,  $P = 0.04$ ) and with the time spent without contact  
358 with the handler ( $R_S = 0.67$ ,  $P = 0.05$ ).

359

360 3.4. Test 4. Behavioural and cortisol responses to isolation and human presence in the test  
361 pen

362 There was no interaction ( $F_{1,30} = 0.00$ ,  $P = 0.97$ ) between the testing condition (isolation  
363 versus human contact) and the treatment (H0 versus H1) on the salivary cortisol  
364 concentration as well as no effect of the testing condition (isolation versus human contact,  
365 log,  $F_{1,30} = 1.29$ ,  $P = 0.27$ ) nor of the treatment (H0 versus H1,  $F_{1,30} = 0.65$ ,  $P = 0.43$ ,  
366 Figure 4).

367 During human presence, more H1 than H0 piglets were observed climbing on the handler (8  
368 versus 1,  $2\hat{I} = 8.36$ ,  $ddl = 1$ ,  $P < 0.01$ ). There was no effect of the treatment on the latency,  
369 duration and number of other behaviours ( $U > 128$ ,  $P > 0.05$ ) whatever the phase of the test.

370

371 4. Discussion

372 4.1. Consequences of being repeatedly stroked and scratched on subsequent responses to  
373 the handler

374 After three weeks of gentle human contact consisting of stroking and scratching, handled  
375 piglets expressed a higher attraction to the handler than control piglets. This was true until at  
376 least six weeks after the beginning of the handling sessions. To our knowledge this is the first  
377 time that scratching was used as a tactile interaction provided by humans. This method  
378 seemed to be a positive way of interacting with animals when associated with stroking. It  
379 remains to determine the relative importance and valence of both tactile stimulations  
380 (scratching and stroking) in the development of the positive relationship in weaned piglets.

381

382 The attraction to the handler was characterized by approaches and contact with her. The  
383 duration of interactions of handled piglets with the handler reached almost two third of the  
384 duration of the test in the home pen and one third of the duration of the test in the test pen,  
385 showing the high interest of piglets for the human handler in both situations. However it  
386 seemed rather low in the arena test (a large pen) compared with the other tests and to  
387 previous studies in comparable conditions (e.g. Hemsworth et al., 1996a; Day et al., 2002).



388 In the home pen, the physical and social environments were familiar to the piglets, and so  
389 piglets probably felt confident in getting in contact with the handler. In the test pen, the space  
390 available was limited, so only fearful piglets stood against the walls of the pen not to be in  
391 contact with the handler. On the contrary, the arena test associated novelty of the  
392 environment and a large space (more than twice the size of the home pen). It took almost  
393 1 min 30 s to the handled pigs to get in contact with the handler, which appears to be higher  
394 than in preceding studies reporting a latency of about 30 s (e.g. Hemsworth et al., 1986b). In  
395 previous studies, individual handling took place where the arena test was performed, and so  
396 handled pigs were used to the experimental environment. This was not the case in our arena  
397 test, which was a novel environment for the piglets. This can easily explain why it took them  
398 more time to get in contact with the human, as the novelty of the situation probably induced  
399 cautious reactions. This is confirmed by the increase in time spent interacting with the  
400 handler between the two phases of the test, both for handled and control piglets. After four  
401 minutes in the arena test, piglets may have been habituated to the environment and thus  
402 may be more confident for interacting with the handler. It can also be hypothesized that  
403 piglets arriving in a new environment are motivated to explore it and that there is a conflict of  
404 interest between the motivation to explore the new environment and to get in contact with the  
405 human with the balance changing over time in favour of the human.

406 We also observed that handled piglets climbed repeatedly on the human. To our knowledge,  
407 this behaviour has never been taken into account *per se* in preceding studies in pigs that  
408 report interactions as a whole. In lambs climbing is a behavioural expression of a higher  
409 attraction to a human handler (Tallet et al., 2009). This behaviour is also expressed in the  
410 mother-young relationship (Keeling and Gonyou, 2001). In our experiment, this could reflect  
411 a search for a more intensive contact with human by the piglets. These behavioural  
412 responses expressed by handled piglets are probably expressions of affinity for the handler  
413 as shown in lambs by Markowitz et al (1998). Thus climbing should be taken into account in  
414 the description of the interactions between pigs and humans.

415

416 Control piglets preferentially expressed reactions characteristics of fear and avoidance (i.e.  
417 being far from the human handler, looking at the handler). Orientation of the head towards a  
418 stimulus is often displayed when an animal faces novel or aversive stimuli (i.e. Nagasawa et  
419 al., 2009; Kanitz et al., 2014 in press). Control animals were not familiar to the handler before  
420 the test sessions, even if they were used to human contact for routine husbandry tasks,  
421 feeding for instance. Consequently the handler could have been perceived as aversive or as  
422 a potential danger, even more that she was a woman while the stockperson was a man. The  
423 gender difference may have strengthened the novelty of the handler. Handled and control  
424 piglets differed in their reaction to the handler, but they did not show any difference in their  
425 behavioural and physiological reactions to social isolation in the different tests. This is in line  
426 with previous studies in domestic chicks or pigs (Jones and Waddington, 1992; Tallet et al.,  
427 2009).

428

429 In addition to behavioural parameters, we compared salivary cortisol level after isolation and  
430 after exposure to the handler. We did not find any significant difference even if the mean  
431 cortisol level seemed to be lower after human presence. There was also no significant effect  
432 of the previous handling treatment on cortisol levels either after isolation or human contact.  
433 This is in agreement with previous studies on low numbers of piglets (Hemsworth et al.,  
434 1986a; Paterson and Pearce, 1992). Introduction in a new environment for an open-field test  
435 is generally sufficient to induce an increase in cortisol level at 10 min after the start of the test  
436 (Zonderland et al., 2009) in pigs. After a stressor, the peak of salivary cortisol is generally  
437 expressed after 15 minutes (Merlot et al., 2011, Ruis et al., 2001). Therefore, the lack of  
438 difference between our treatments cannot be explained by a duration not sufficient to  
439 measure an adrenal response. Alternatively, it can be hypothesized that the situation itself,  
440 i.e. being taken away from the group, carried in a cart and put in an unfamiliar environment  
441 was stressful enough to induce a high adrenal response that would have masked the  
442 possible effect of the previous experience with the handler. Indeed, salivary concentrations  
443 measured in the pigs from the present experiment (about 4 ng/ml) are much higher than

444 those observed, with the same method, in growing pigs in their home pen (about 1 ng/ml)  
445 (Merlot et al., 2012).

446

#### 447 *4.2. Perception of the different types of gentle tactile contact in the test pen (Test 3)*

448 During the isolation phase of the test pen, vocalisations and heart parameters (heart rate,  
449 RMSSD) were similar in handled and control piglets. The heart rate was high at the  
450 beginning of the test since it was around 175 bpm while resting heart rate of weaned piglets  
451 is about 130 bpm (Talling et al., 1996; Imfeld-Mueller et al., 2011). This may be due to the  
452 stress induced by the experimental procedure (transport, isolation from congeners, arriving in  
453 a new environment, fitting the recording device under a belt) even though care was taken to  
454 habituate the pigs to this experimental procedure as far as possible.

455

456 Once the handler entered the pen and sat down, handled piglets approached her more  
457 readily than control ones, climbed on her more frequently like in the other tests. They also  
458 expressed more high-pitched vocalisations which are indicators of stress in pigs (Weary et  
459 al., 1999; Schön et al., 2004; Tallet et al., 2013). These vocalisations could result from  
460 frustration due to the fact that the handler did not provide gentle tactile contact as expected  
461 by the handled piglets. This is supported by the fact that they did not express high-pitched  
462 vocalisations in the other phases of the test when they were touched. Terlouw and Porcher  
463 (2005) showed that repeated refusal of a stockperson to with positive tactile contact when  
464 they touch him progressively induces frustration behaviours as indicated by increased levels  
465 of locomotion, rubbing, immobility, and snout contact with the wall.

466 The arrival of the handler induced variations in the cardiac activity. Indeed, the heart rate  
467 during the first 10 s of presence of the handler was higher than the heart rate during the last  
468 10 s of isolation for piglets of both treatments. Therefore, the arrival of the handler probably  
469 triggers excitement in piglets. In handled piglets, this excitement could be related to the  
470 expectation of positive tactile stimulation whereas in control piglets it is probably related to  
471 fear. Indeed, an increase in heart rate is often found in tests evaluating fear reactions

472 (Forkman et al., 2007). This is supported by the observation that control piglets spent more  
473 time without contact with the handler. Moreover, in those piglets, the correlation between the  
474 time spent away from the handler and the heart rate was positive and significant.

475

476 During the test, we observed time-related variations of the cardiac activity that are similar in  
477 both groups and suggest an overall similar perception of tactile contact regardless of the  
478 previous exposure to this contact. We demonstrated a general decrease of the heart rate that  
479 was similar in both treatments and was significant between the fourth and fifth phases of the  
480 test. We expected the heart rate to be lower after human arrival or during tactile stimulations  
481 in handled than in control piglets but this was not the case. One hypothesis is that stroking  
482 and scratching are spontaneously perceived as positive by piglets, and so induce the  
483 development of positive reactions along the test duration. Another hypothesis is that there is  
484 a very fast habituation to this novel gentle tactile stimulation by control piglets. This is  
485 supported by the fact that during the first phase of stroking, the control piglets spent more  
486 time without contact with the handler than handled piglets but afterwards this level was  
487 similar. The pre-exposure of control piglets to the handler in the preceding tests could have  
488 facilitated the process. However, the results have to be taken carefully due to the fact that  
489 the order of the phases was exactly the same for all animals, and thus the effect of the type  
490 of interaction is confused with the habituation to the testing conditions. Despite the absence  
491 of difference in heart rate, we observed different profile for the heart rate variability (i.e.  
492 RMSSD). It decreased till the phase of scratching the head, increased afterwards in control  
493 piglets whereas it continued to decrease in handled piglets. RMSSD is known to increase in  
494 positive situations (Mohr et al., 2002; Reefmann et al., 2009b). This would mean that being  
495 scratched at the rear was perceived as positive by control piglets, but not by handled piglets.  
496 This is the first time that the effect of scratching was studied in piglets. Control piglets may  
497 have perceived this type of interactions given by the human as positive; while handled piglets  
498 would have developed habituation and absence of reaction.

499

500 Despite the differences in the cardiac activity, there was no clear behavioural expression of  
501 the differentiation between the two groups of pigs for scratching the rear. However, we did  
502 not look at more subtle behavioural expression of emotions, like tail movements or ear  
503 positions (Reefmann et al., 2009a) and differences in such parameters cannot be excluded.  
504 Another explanation is that, in many cases, the cardiac activity varies before observable  
505 behavioural modifications (von Borell et al., 2007), and thus the 2-min phases may have  
506 been too short to observe behavioural variations. This suggests that the cardiac activity is a  
507 very interesting tool to assess human-animal relationship and responses to precise  
508 categories of contact.

509

## 510 5. Conclusion

511 Providing both stroking and scratching to piglets for three weeks after weaning induced  
512 attractive reactions towards the handler both in their home pen and in an unfamiliar  
513 environment, revealing that they have developed an affinity towards the handler. The  
514 comparison of the heart rate activity between handled and control piglets did not provide any  
515 evidence of a different perception of these contacts according to the previous handling  
516 experience of the piglets. Piglets would be sensitive to human stroking and scratching in  
517 such way that these contacts are positively perceived by the animals even at the first  
518 exposure. Differences in the sensitivity of the different body regions will have to be further  
519 investigated.

520

## 521 Acknowledgements

522 This project was funded by the INRA PHASE division (Crédit incitatifs 2009). The authors  
523 thank Patrick Touanel, Michel Lefèbvre, Carole Guérin, Fabien Guérin and Xavier Averós for  
524 their active involvement in the experiment.

525

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673

674 Figures captions

675 Figure 1. Mean ( $\pm$  sem) time spent near the handler and looking at the handler for control  
676 (H0, n=16, light grey) and handled (H1, n=15, hatched) piglets during phases 1 and 3  
677 (human presence) of Test 2.

678 For “near the handler”, the interaction between treatment and phase is not significant ( $P >$   
679 0.1). The effects of phase or treatment are shown by asterisks: \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ .

680 For “looking at handler”, the interaction between treatment and phase is significant ( $P <$   
681 0.05). Comparisons between means are shown by letters a,b,c: values with different letters  
682 differ significantly ( $P < 0.05$ ).

683

684 Figure 2. Change in median ( $\pm$  quartiles) (a.) number of low-pitched vocalizations, (b.) time  
685 spent investigating the handler (s), and (c.) time spent without contact with the handler (s)  
686 according to the treatment (light grey: control = H0, and black: handled = H1) and the phases  
687 of Test 3 (Hsat = handler immobile, Strok1 and 2 = handler stroking the piglets, ScratH =  
688 handler scratching the head of the piglet, ScratR = handler stroking the piglet at its rear).  
689 Treatment effect by phase: t:  $0.05 < P < 0.1$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

690 Phase effect within a treatment: values with different letters differ significantly ( $P < 0.05$ );  
691 a,b,c (black) correspond to H1 treatment, and d,e,f (grey) to H0 treatment.

692

693 Figure 3. Change in mean ( $\pm$  sem) heart rate (a.) and RMSSD (b.) according to the treatment  
694 (light grey: control = H0, and black: handled = H1) and the phases of Test 3 (Alone= piglet  
695 alone, Hsat = handler immobile, Strok1 and 2 = handler stroking the piglets, ScratH = handler  
696 scratching the head of the piglet, ScratR = handler stroking the piglet at its rear).

697 Treatment effect by phase: \*\*:  $P < 0.01$ .

698 Phase effect: values with different letters differ significantly ( $P < 0.05$ ).

699 A,B correspond to phase effect for both treatments, a,b,c (black) correspond to H1 treatment,  
700 and d,e,f (grey) to H0 treatment.

701

702 Figure 4. Mean ( $\pm$  sem) salivary cortisol concentration (ng / ml) of control piglets (H0) and  
703 handled piglets (H1) after a period of 15 min of isolation or 15 min of human contact (Test 4).

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2 Tables

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4 Table 1. Timeline of the experiment

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Day of the experiment	Pigs' age in days ( $\pm 2$ )	Test	Period	Event / observation	Place	Observation
0	28		Morning	Weaning		
1 - 25	29 - 53		Morning + afternoon	Sessions of human contact	Home pen	-
25	53		Afternoon	Reaction to human presence	Home pen	Behaviour
26-27	54-55	1	Morning	Reaction to human presence and departure	Arena test	Behaviour
32	60	2	Morning + afternoon	Two sessions of human contact	Home pen	-
33-35	61-63	3	Morning	Reaction to human contact	Test pen	Behaviour and cardiac activity
			Afternoon	One session of treatment	Home pen	-
40-43	68-71	4	Afternoon	Reaction to isolation and human presence	Test pen	Behaviour and salivary cortisol

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Table 2. Behavioural observations during the different tests of the experiment

<b>behaviour</b>	<b>description</b>	<b>used in tests</b>
<b>Interactions with the handler</b>		
Look at the handler	Head directed to the handler	1, 2
Investigation of the handler	Head of the piglet in contact with the handler (touching or sniffing)	1, 2, 3, 4
Body contact	Animal standing and in contact (any part of the body except the head) with the handler	1, 3, 4
Body contact lying	Animal lying down or sitting (any part of the body except the head) in contact with the handler	3, 4
Climb	The piglet climbs on the handler, with at least its front legs on the thighs of the handler	1, 2, 3, 4
<b>Vocalisations</b>		
High-pitched vocalizations	Screams, squeals and grunt-squeals	2, 3
Low-pitched vocalizations	grunts	2, 3
<b>Locomotion</b>		
Number of zones crossed	Total number of zone crossed during the test. The animal was considered to enter a zone when its two front legs were in the zone.	2
<b>Other behaviours</b>		
Bucket contact	In contact with the bucket on which the handler is seated	1
No contact	No contact with the handler	1, 3
Avoidance	The piglet moves away from the handler, or turns its head opposite to the handler	1
Escape	Attempt to escape from the pen, by jumping against the walls	2, 3, 4

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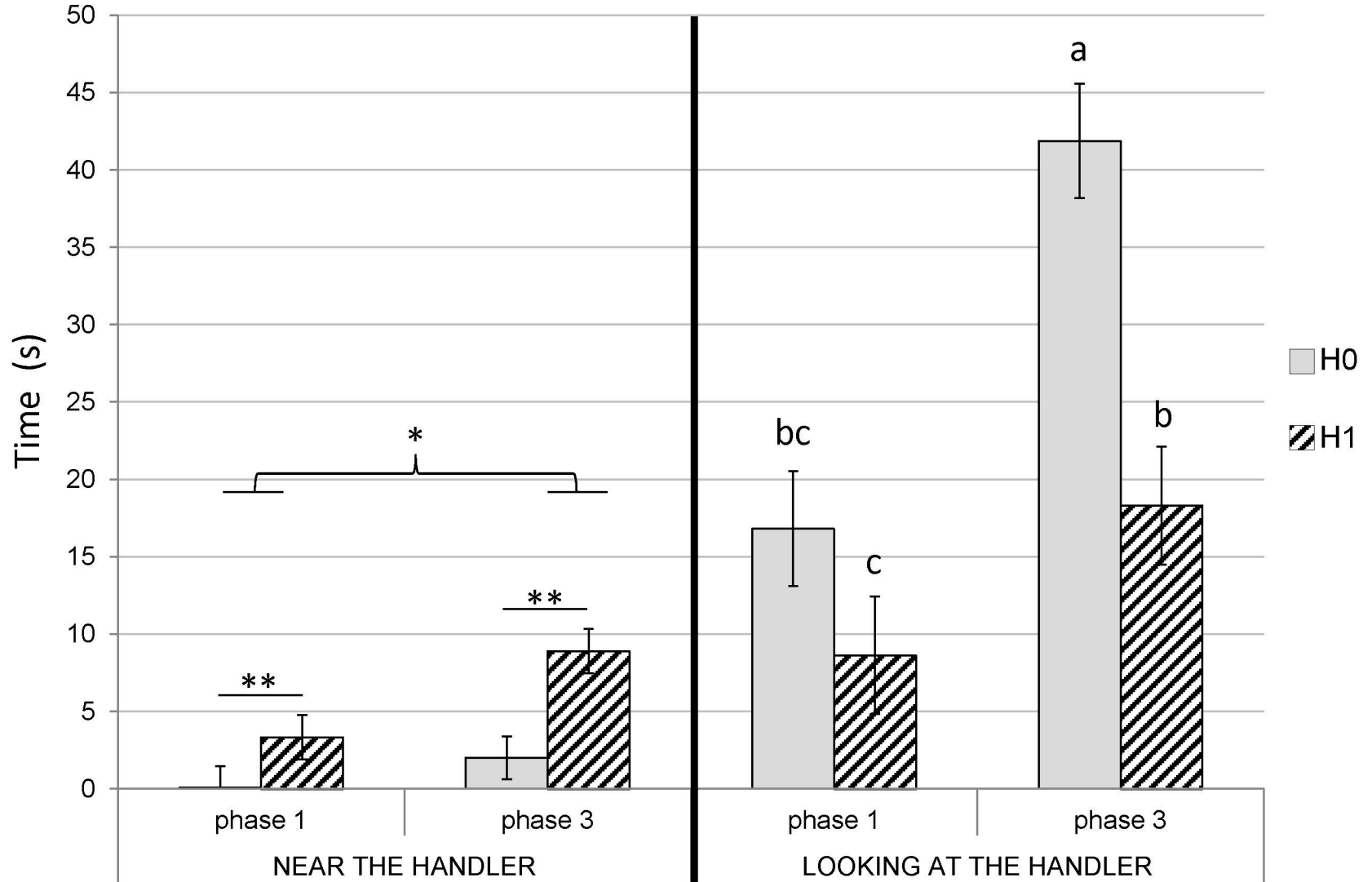
Table 3. Evolution of the mediane (IQ) heart rate (beat per minute) of the piglets according to the behaviour of the handler during the different phases of Test 3 (Hsat = handler immobile, Strok1 and 2 = handler stroking the piglets, ScratH = handler scratching the head of the piglet, ScratR = handler stroking the piglet at its rear).

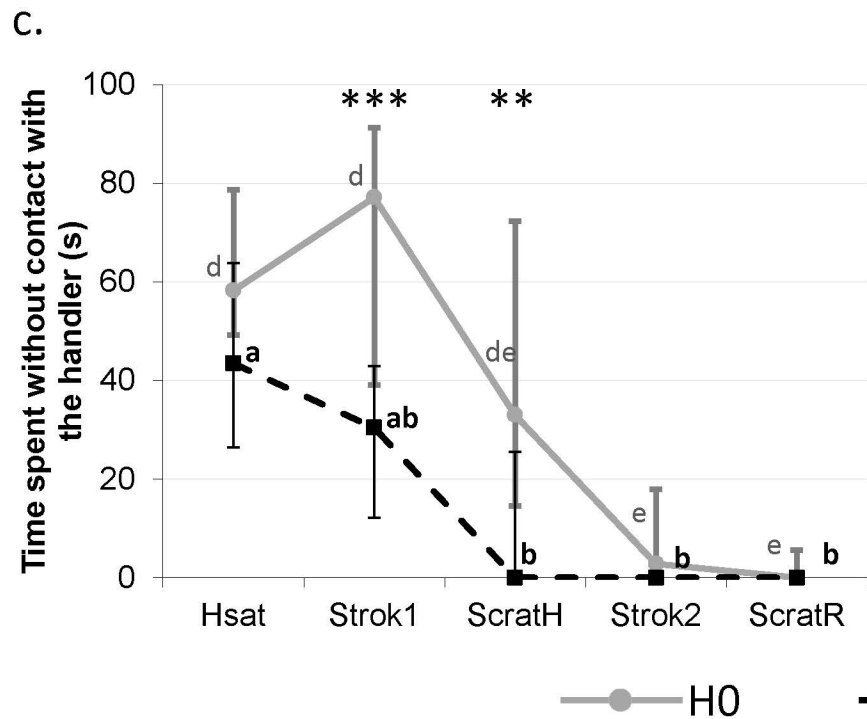
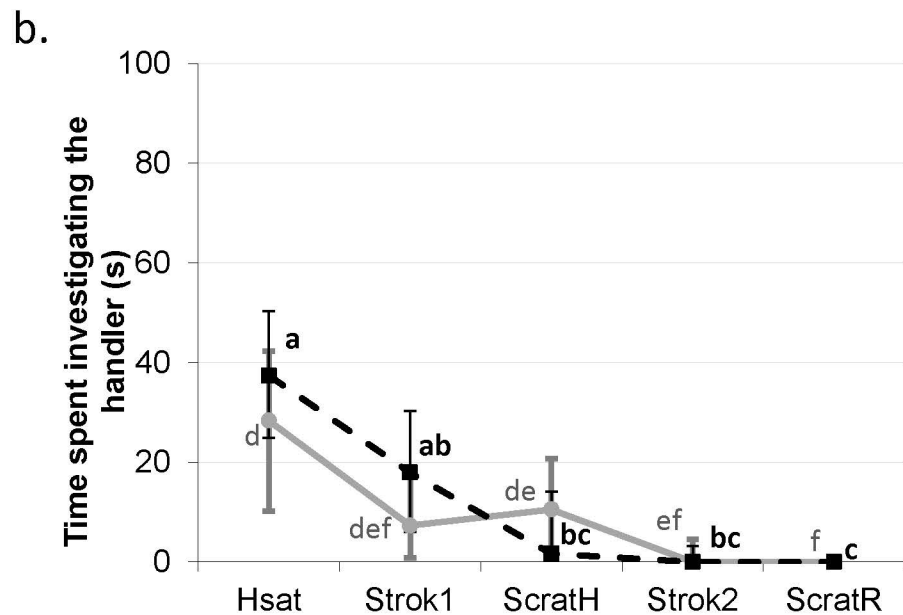
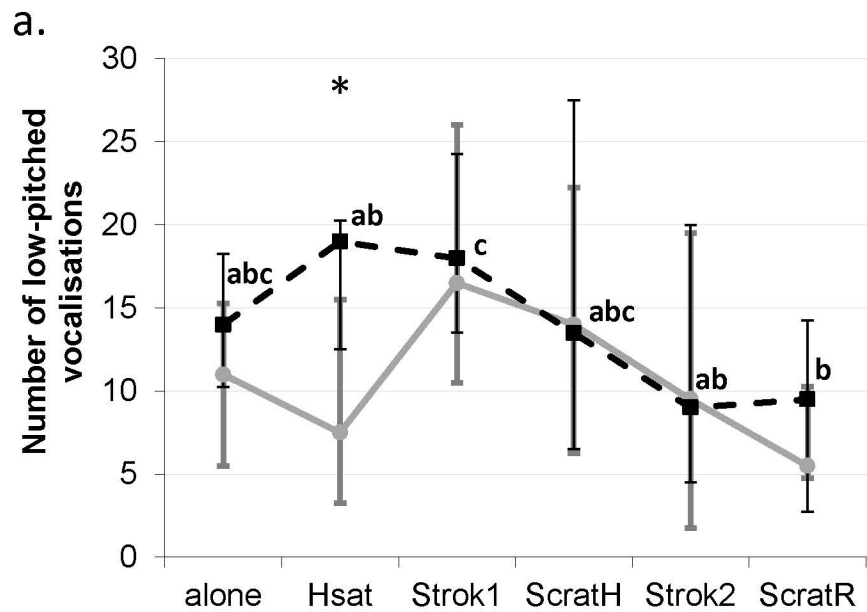
phase of test	variation	H0 treatment			H1 treatment			
		<i>N</i>	<i>z</i>	<i>P</i>	variation	<i>N</i>	<i>z</i>	<i>P</i>
between Alone and Hsat	26 (46)	10	<b>2.09</b>	<b>0.04</b>	15 (25)	8	<b>2.10</b>	<b>0.04</b>
between Hsat and Strok1	-5 (10)	10	1.48	0.14	-5 (15)	8	1.40	0.16
between Strok1 and ScratH	-1 (8)	10	0.56	0.58	0 (14)	7	0.85	0.40
between ScratH and Strok2	-4 (4)	10	1.78	0.07	<b>-5 (5)</b>	9	<b>2.19</b>	<b>0.03</b>
between Strok2 and ScratR	-2 (3)	11	0.98	0.33	<b>-5 (4)</b>	10	<b>2.29</b>	<b>0.02</b>

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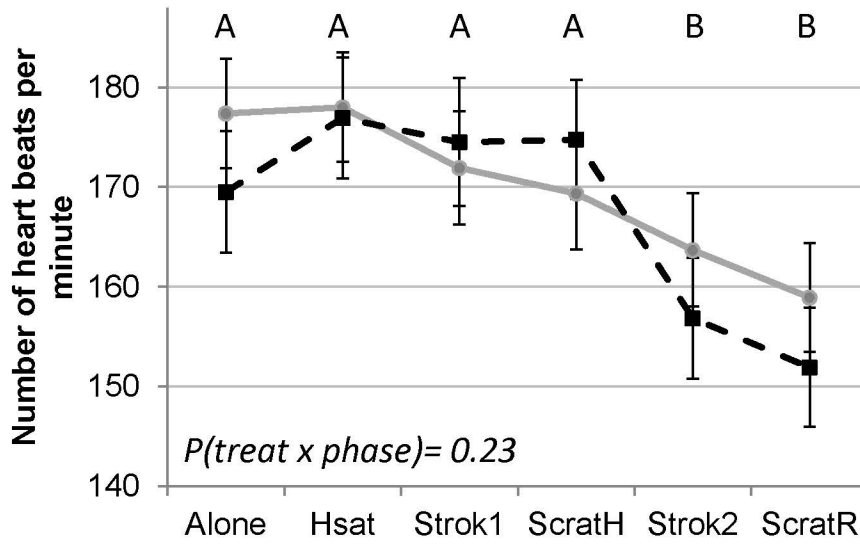
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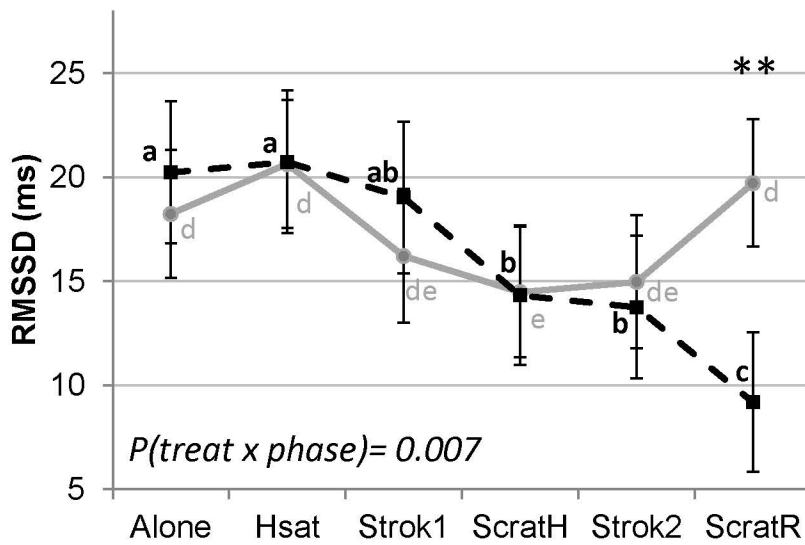




a.



b.



—●— H0

- -■- - H1

Cortisol concentration (ng / ml)

