

23 **Abstract**

24

25 Emotions are important because they enable the selection of appropriate behavioural
26 decisions in response to external or internal events. Techniques for understanding and
27 assessing animal emotions, and particularly positive ones, are lacking. Emotions can be
28 characterized by two dimensions; their arousal (bodily excitation), and their valence
29 (negative or positive). Both dimensions can affect emotions in different ways. It is thus
30 crucial to assess their effects on biological parameters simultaneously, so that accurate
31 indicators of arousal and valence can be identified. In order to find convenient and non-
32 invasive tools to assess emotions in goats (*Capra hircus*), we measured goat physiological,
33 behavioural and vocal responses in four situations: 1) control situation (no external
34 stimulus, neutral); 2) anticipation of a food reward (positive); 3) food-related frustration
35 (negative); 4) isolation away from conspecifics (negative). These situations were
36 characterised by different levels of arousal, assessed *a posteriori* by heart rates measured
37 during the tests. We found several clear, reliable indicators of arousal and valence. During
38 situations of higher arousal, goats had lower heart-rate variability (RMSSD) and higher
39 respiration rates. They displayed more head movements, moved more, had their ears
40 pointed more often forward and less often on the side (horizontal), and produced more
41 calls. They also produced calls with higher fundamental frequencies and higher energy
42 distribution. In positive situations, goats had their ears less often orientated backward
43 and spent more time with their tails up than in negative situations. Furthermore, they
44 produced calls in which the fundamental frequencies were less variable. Our methods for
45 assessing the effects of emotional arousal and valence on biological parameters could lead
46 to more effective monitoring and understanding of animal emotions, as well as to a
47 better understanding of the evolution of emotions through cross-species comparisons.

48

49 **Keywords.** Acoustic communication, arousal, *Capra hircus*, positive emotions, ungulates,
50 valence, vocal analysis

51 INTRODUCTION

52

53 Although the existence of animal emotions has been suggested since Darwin (1872),
54 techniques for understanding and assessing these affective states, and particularly
55 positive ones, are still lacking. The discovery of clear emotional indicators is crucial for
56 many disciplines, including animal behaviour, neuroscience, psychopharmacology and
57 animal welfare (Mendl, Burman, & Paul, 2010). Emotions are composed of four
58 components: neurophysiological, behavioural, cognitive and subjective (Keltner & Lerner,
59 2010). While there is evidence for a subjective, conscious component of emotions only in
60 humans, the other components can potentially be used as indicators in non-human
61 animals (Mendl et al., 2010).

62 Unlike the “discrete emotion approach”, which suggests the existence of a small
63 number of fundamental emotions, the “dimensional approach” proposes to characterize
64 emotions according to their two main dimensions: arousal (bodily activation or excitation;
65 e.g. calm versus excited) and valence (negative or positive; e.g. sad versus happy;
66 Russell 1980). This approach is very promising for the study of animal emotions (Mendl
67 et al., 2010). Its recent use has allowed substantial progress to be made in identifying
68 animal behavioural (e.g. pigs, *Sus scrofa*, Imfeld-Mueller, Van Wezemael, Stauffacher,
69 Gyax, & Hillmann, 2011; review, Murphy, Nordquist, & van der Staay, 2014; sheep,
70 *Ovis aries*, Reefmann, Bütikofer Kaszàs, Wechsler, & Gyax, 2009a), physiological (e.g.
71 hens, *Gallus domesticus*, Davies, Radford, & Nicol, 2014; sheep, Reefmann, Bütikofer
72 Kaszàs, Wechsler, & Gyax, 2009b) and cognitive indicators of emotional arousal and
73 valence (goats, Briefer & McElligott, 2013; rats, *Rattus norvegicus*, Burman, Parker, Paul,
74 & Mendl, 2008; review, Mendl, Burman, Parker, & Paul, 2009). In addition, the
75 relationship between an individual’s inner state and the vocalisations it produces suggests
76 that vocalisations are promising indicators of emotions (Briefer, 2012; Manteuffel, Puppe,
77 & Schön, 2004).

78 Indicators of animal emotional arousal have been extensively studied in negative
79 situations (e.g. stress, fear in farm animals, Forkman, Boissy, Meunier-Salaün, Canali, &

80 Jones, 2007). Conversely, studies of arousal indicators during situations of positive
81 valence are rare. Indicators that could allow us to differentiate between negative and
82 positive situations (i.e. valence indicators) have also been poorly studied. Finding
83 indicators of valence requires comparing animals that are exposed to negative versus
84 positive situations. Yet, changes in parameter values between neutral and negative
85 situations are often easier to detect than between neutral and positive situations,
86 because negative emotions often trigger higher arousal levels than positive ones (Boissy
87 et al., 2007). Another concern regarding research on indicators of emotions is that very
88 few studies have investigated both arousal and valence in a given species (but see
89 Gogoleva et al., 2010; Soltis, Blowers, & Savage, 2011). Additionally, the emotional
90 situations that are used often differ in both dimensions simultaneously, or may differ in
91 more than simply the emotions they trigger (e.g. comparing the effect of pain as a
92 negative situation versus food reward as a positive one). This results in confusion about
93 which dimension affects the measured parameters. More precise arousal indicators could
94 assist in identifying and thus minimizing stress during negative situations, while more
95 accurate valence indicators could allow us to distinguish between negative and positive
96 situations. This would then lead to enhanced animal wellbeing by promoting situations
97 that trigger positive emotions (Boissy et al., 2007).

98 In this study, we investigated indicators of both emotional arousal and valence in
99 domestic goats. Goats are highly social and vocal animals that in the wild (feral goats) live
100 in complex fission-fusion societies (Stanley & Dunbar, 2013). This species should benefit
101 from behavioural or vocal expression of emotions, as a mean to regulate social
102 interactions within groups (Panksepp, 2009). Goats have good cognitive abilities, such as
103 perspective taking (Kaminski, Call, & Tomasello, 2006) and conspecific gaze following
104 (Kaminski, Riedel, Call, & Tomasello, 2005). They have the ability to use indirect
105 information (i.e., the absence of food; Nawroth, von Borell, & Langbein, 2014b) and
106 human pointing and touching cues (Nawroth, von Borell, & Langbein, 2014a) to find a
107 reward. They also have good visual discrimination learning abilities (Langbein, Nürnberg,
108 & Manteuffel, 2004) and long-term memory (Briefer, Haque, Baciadonna, & McElligott,

109 2014; Briefer, Padilla de la Torre, & McElligott, 2012). The most common goat
110 vocalisation is the contact call, which is used to maintain contact at relatively close
111 distance (Briefer & McElligott, 2011a). Goats produce two kinds of contact calls: closed-
112 mouth contact calls and open-mouth contact calls (Ruiz-Miranda, Szymanski, & Ingals,
113 1993). Contact calls contain information about individuality (Briefer & McElligott, 2011a),
114 age, sex and body size (Briefer & McElligott, 2011b), kinship and even group
115 membership of the producer (Briefer & McElligott, 2012). Playback experiments have
116 shown that these vocalisations allow mothers and kids to recognize each other from at
117 least one week postpartum (Briefer & McElligott, 2011a), and that mother goats
118 remember the calls of their kids for up to one year after separation (Briefer et al., 2012).
119 Goat behaviour and vocalisations have been shown to be affected by the degree of social
120 isolation (complete or partial), suggesting the existence of indicators of negative arousal
121 (Siebert, Langbein, Schön, Tuchscherer, & Puppe, 2011). In terms of potential indicators
122 of valence, goat patterns of behaviour, sympathetic reactions and brain activity have
123 been shown to differ between positive and negative situations (i.e. different valence;
124 Gygax, Reefmann, Wolf, & Langbein, 2013). In this study, we assessed physiological,
125 behavioural and vocal profiles linked to both arousal and valence, by testing which
126 dimension was most responsible for changes in the measured parameters.

127 We combined new frameworks recently adapted from humans to animals to
128 analyse vocalisations (source-filter theory; Taylor and Reby 2010), and emotions (Mendl
129 et al., 2010), in order to find non-invasive indicators of emotions in goats. We placed goats
130 in four situations likely to induce different emotional arousal and valence: control
131 (neutral), anticipation of food reward (positive), food frustration (negative) and social
132 isolation (negative). Physiological stress (nonspecific response of the body to any
133 demand made upon it), and thus heart rate, increases with arousal, whether the situation
134 is positive or negative (Seyle, 1976). For this reason, we assessed the arousal triggered
135 by our experimental emotional situations by comparing the heart rates of goats in
136 response to the tests. In the absence of well-established valence indicators in the
137 literature, we inferred the valence of our situations based on knowledge of the function of

138 emotions and on goat behaviour. We tested the hypothesis that emotional arousal and
139 valence in goats are indicated by particular physiological, behavioural and vocal profiles.
140 For instance, we expected physiological parameters linked to the autonomic nervous
141 system (e.g. heart rate and respiration rate) to be affected by arousal, while behavioural
142 and vocal parameters could indicate both dimensions (Briefer, 2012; Imfeld-Mueller et
143 al., 2011; Reefmann, Wechsler, & Gyax, 2009). We defined the parameters that
144 changed according to increased arousal levels as reliable indicators of arousal. Similarly,
145 we defined the parameters that changed consistently from negative to positive valence
146 as reliable indicators of valence.
147

148 **METHODS**

149

150 *Subject and Management Conditions*

151

152 The study was carried out at a goat sanctuary (Buttercups Sanctuary for Goats,
153 <http://www.buttercups.org.uk>), Kent (UK). We tested 22 adult goats (11 females and 11
154 castrated males), which were fully habituated to human presence and could be led
155 around using a lead rope (Baciadonna, McElligott, & Briefer, 2013; Briefer & McElligott,
156 2013), between May and June 2011. They were aged 3-17 years and of various breeds
157 (Table 1). They had been at the sanctuary for at least 2 years (range = 2-11 years). Five
158 of these goats (three females and two males) had been rescued and brought to the
159 sanctuary because of poor welfare conditions (three goats) or because they had been
160 found abandoned (two goats). These five goats had been at the sanctuary for at least 4
161 years in 2011. The other goats had been housed in good conditions and were brought to
162 the sanctuary because their owners could not keep them anymore.

163 All goats at our study site are released into a large field during the day. At night,
164 they are kept indoors in individual or shared pens (2 or 3 goats, average size = 3.5 m²)
165 with straw bedding, within a larger stable complex. Routine care of the animals is
166 provided by sanctuary employees and volunteers. Goats have ad libitum access to hay,
167 grass (during the day) and water, and are also fed with a commercial concentrate in
168 quantities according to their state and age. Every stable is cleaned on a daily basis. All
169 goats are inspected each day by the sanctuary employees and volunteers, and are
170 checked regularly by a vet and given medication when appropriate.

171

172 *Situations inducing Emotional States*

173

174 We designed three brief situations (≤ 5 min) of various arousal and valence, which were
175 likely to elicit vocalisations by the goats (anticipation of food reward (positive), food
176 frustration (negative) and social isolation (negative)). In addition, these situations were

177 compared to a neutral, control situation. To avoid stress linked to isolation, goats were
178 tested in familiar pairs (identical during the whole experiment) during the control,
179 anticipation of food reward and food frustration situations, but not during the isolation
180 situation. When tested in a pair, the two goats were located in adjacent pens. They could
181 thus hear and see each other, but not physically interact.

182 We introduce the experimental situations here and give more details about the
183 procedure in the next section (*Experimental Procedure*). i) During the control situation,
184 goats were left unmanipulated in a pen with hay ("Control"). This situation did not elicit
185 any calls, but allowed us to obtain baseline values for physiological and behavioral data.
186 ii) The positive situation was the anticipation of an attractive food reward that the goats
187 had been trained to receive during three days of habituation ("Feeding"). iii) After goats
188 had been tested with the Feeding situation, they were tested with a food frustration
189 situation. This consisted of giving food to only one of the goats in the pair and not to the
190 subject ("Frustration"). iv) The second negative situation was brief isolation, out of sight
191 from conspecifics behind a hedge. For this situation, goats were tested alone and not in a
192 pair ("Isolation").

193

194 *Determination of Arousal Levels and Valence of the Situations*

195

196 The relative level of activity of the autonomic nervous system is determined to a great
197 extent by current emotions (von Borell et al., 2007). Heart rate is a well-recognized
198 indicator of physiological stress (e.g. Forkman et al., 2007), which is linked to emotional
199 arousal during situations of both positive and negative valence (Seyle, 1976). Therefore,
200 to determine arousal levels, we assessed between-situation differences in heart rate. By
201 contrast, no clearly established indicator of emotional valence exists for goats or related
202 mammals in the literature, because studies that have investigated both emotional
203 arousal and valence simultaneously (in order to highlight clear indicators of valence that
204 are not influenced by arousal) are rare. The valence of our situation was thus inferred
205 from knowledge of the function of emotions and of goat behaviour.

206 Positive emotions result from encounters with rewarding stimuli that enhance
207 fitness. They trigger approach behaviour towards the reward (Mendl et al., 2010).
208 Negative emotions result from encounters with punishing stimuli that threaten fitness,
209 and they trigger avoidance behaviour (Mendl et al., 2010). Accordingly, situations that
210 involve a stimulus that can enhance fitness (e.g. food reward) and that an animal would
211 want to approach can be assumed to be positive. By contrast, situations that involve a
212 stimulus that somehow threaten fitness and that an animal would try to avoid can be
213 assumed to be negative.

214 The Control situation was assumed to be neutral. There is some evidence
215 suggesting that appetitive-anticipation of a reward induces positive emotional states (e.g.
216 satisfaction/contentment; Boissy et al., 2007; Moe et al., 2009; Spruijt, van den Bos, &
217 Pijlman, 2001). We thus assumed the Feeding (anticipation of food reward) situation as
218 positive for goats, because this situation would enhance fitness through the acquisition of
219 a food reward. However, failure to obtain expected food is likely to lead to a high-arousal
220 negative state (Mendl et al., 2010; Rolls, 2005). The Frustration situation was therefore
221 considered negative for the goats, because this situation would (in the wild at least)
222 threaten fitness through the lack of food intake. Finally, isolation (even during a short
223 period) for social herd-living animals such as goats, can be stressful (Price & Thos, 1980;
224 Siebert et al., 2011). Consequently, we considered the Isolation situation to be also
225 negative for goats, because it could potentially threaten fitness through greater exposure
226 to predators.

227

228 *Technique used for Data Collection*

229

230 We collected physiological data using a small wireless non-invasive monitor, fixed to a
231 belt placed around the goat's chest (MLE120X BioHarness Telemetry System, Zephyr).
232 For detailed behavioural analyses, all tests were video recorded using a Sony DCR-SX50E
233 camcorder. Finally, vocalisations were continuously recorded during the tests at distances
234 of 3-5 m from the vocalising animal using a Sennheiser MKH-70 directional microphone

235 (frequency response 50 – 20.000 Hz; max SPL 124 dB at 1 kHz), connected to a Marantz
236 PMD-660 numeric recorder (sampling rate: 44.1 kHz).

237

238 *Experimental Procedure*

239

240 During the Control, Feeding and Frustration situations, goat pairs were tested in two
241 indoor adjacent pens, 3 m² each, within a familiar larger stable complex. During the
242 Isolation situation, they were tested individually in a 3.4 m² and 2.10 m high outdoor pen
243 made of gaited hurdles, in a familiar field (usual daytime range) out of sight from other
244 goats. To minimize stress linked to novelty, the emotional tests were preceded by three
245 days of habituation. During these three days, goats were gradually habituated to the set-
246 up, to the measurement equipment (i.e. wireless non-invasive monitor) and to the
247 Feeding and Isolation situations (both repeated once per day, during the three
248 habituation days). They were not habituated to the Control situation (everyday
249 situation), nor to the Frustration situation, in order to ensure that food was always
250 expected during the Feeding situation. Goats were then tested over three days with one
251 test per day (i.e. Feeding, Frustration and Isolation situations).

252 i) The Control situation (no habituation) was carried out on two consecutive days.
253 On each of these two days, we placed the pair of goats in adjacent pens for 5 min, with
254 hay in the feeders, and left them undisturbed. ii) For the Feeding situation, each pair of
255 goats was placed in the indoor pens for 5 min pre-test with hay in the feeders. At the end
256 of the 5 min pre-test, during the first habituation day, an experimenter (same person
257 throughout the experiment) presented two buckets with food (commercial concentrate
258 for goats with fresh chestnut tree leaves) simultaneously to the two goats for 1 min,
259 before giving them the food. During the second and third habituation days, and during
260 the test day, the hay was removed for 2 min before the food presentation, in order to
261 increase food motivation. Then, the experimenter presented the food simultaneously to
262 the two goats for 20 s, walked outside the stable complex for 1 min, and walked back
263 towards the goats while shaking the food bucket to make it obvious, and presented the

264 food again for 20 s. Then, he walked away a second time for 1 min, walked back and
265 gave them the food. This allowed us to obtain three events (before the first and second
266 20 s presentation and before giving them food), when the experimenter approached the
267 goats with food, resulting in the production of vocalisations. iii) The Frustration situation
268 (no habituation) started in the same way as the Feeding situation, with 5 min pre-test
269 (with hay), followed by 2 min during which the hay had been removed. Then, the
270 experimenter presented the food buckets (similar buckets and food as during the Feeding
271 situation) during 20 s simultaneously to the two goats and gave food to only one of the
272 goats in the pair, while the subject did not receive food. This lasted for 4 min, after which
273 the experimenter also gave food to the subject. iv) To habituate the goats to the
274 Isolation situation, goats were placed in pairs during the first two habituation days, in
275 two identical and adjacent pens, out of sight of the other goats, for 5 min. On the last
276 habituation day, the adjacent pen was removed and each goat was placed in the test pen
277 alone. During the test day, the Isolation situation started with 5 min pre-test, during
278 which the subject was placed in the indoor pen used for the other situations, with hay
279 and with the paired goat in the adjacent pen. This allowed the subject to settle down
280 after it was equipped with the heart-rate monitor. Then, the subject was placed alone in
281 the outdoor isolation pen and left there for 5 min. At the end of the 5 min, it was
282 returned to the other goats.

283 The 22 goats were tested in every situation (repeated measure design). During
284 habituation days, goats were placed in both the Feeding and Isolation situations each day
285 in a random order. During the three test days (one test per day), the order of the
286 emotional situations was pseudo-random. The only constraint was that, for each
287 individual, the Feeding situation always preceded Frustration situation (1-6 days before),
288 in order to ensure a positive state during the Feeding situation. Within a pair, both goats
289 were tested with the Control and Feeding situations at the same time. This prevented
290 food frustration if one of the goats was not simultaneously tested. They were tested with
291 the two other tests (Frustration and Isolation) on different days for each goat in the pair.
292 The Control situation was carried out on different days as the emotional situations.

293

294 *Response Measures*

295

296 *Physiological measures*

297 We measured heart rate and respiration rate, which are likely to be affected by emotions
298 (Reefmann et al., 2009b; von Borell et al., 2007), and are part of the of the stress
299 response of the Sympatho-Adreno-Medullary axis (SAM; i.e. neuronal or “fight-or-flight”
300 response), as opposed to the stress response of the second main stress axis, the
301 Hypothalamo–Pituitary-Adrenocortical axis (HPA; i.e. humoral response). Because
302 vocalisations are affected by the autonomic nervous system (ANS; Scherer, 1986, 2003),
303 vocal parameters are more likely to be correlated with the SAM response indicators than
304 the HPA response indicators (e.g. cortisol), at least over short time scales (Schrader &
305 Todt, 1998). Before the beginning of the experiment, we quickly clipped a small patch of
306 hair below the heart-rate monitor, in order to improve the contact between the
307 electrodes and the body. Goats were equipped with the monitor before each situation, on
308 both the habituation and test days. This technology was also removed immediately after
309 each test. ECG gel was applied on the parts of the belt containing the electrodes before
310 each use. The data (continuous ECG trace and breathing wave, i.e. inhalation/exhalation
311 cycle) were then transmitted and stored in real time to a laptop using LabChart software
312 v.7.2 (ADInstrument) for later analyses. During the tests, one experimenter, who was
313 concealed in a pen close to the subjects, quietly recorded comments into the software
314 indicating important events (e.g. when the other experimenter was presenting food to
315 the goats, leaving or coming back during Feeding situation, or when the paired goat was
316 given food and finished eating during the Frustration experiment). This allowed us to
317 measure physiological parameters at the exact times when these events occurred.

318 When possible (i.e. good quality signal; clearly visible heart beats on the ECG
319 trace and respiration on the breathing wave), we analysed data for each situation over
320 three 10 s sections, in which the software could track the heart beats (ECG trace) and
321 the inspiration–exhalation cycles (breathing wave) accurately (Reefmann, Wechsler, et

322 al., 2009: mean \pm SD for each section: Control, 10.03 ± 0.03 s; Feeding, 9.26 ± 0.19 s;
323 Frustration, 9.83 ± 0.10 s; Isolation, 9.69 ± 0.14 s). For the Control situation, the three
324 sections were collected at the beginning, middle and end of the time starting 1 min after
325 the tested pair of goats was placed in the adjacent pens with hay in the feeder. This
326 allowed the goats to settle down before we started data collection. For the Feeding
327 situation, the first section corresponded to the time just before the first food
328 presentation, the second section to the time when the experimenter came back with the
329 food for the first time (i.e. after opening the door to enter the stable complex), and the
330 third section to the time when the experimenter came back with the food for the second
331 time (i.e. after opening the door to enter the stable complex and just before giving the
332 food). The data were thus collected only when the subject could hear/see the
333 experimenter coming towards them with food, and was likely to be experiencing a
334 positive state. This also ensured that our data would reflect an anticipatory state, as
335 opposed to a consummatory state while feeding or a post-consummatory state after
336 feeding, which are likely to be of different arousal (Spruijt et al., 2001). For the
337 Frustration situation, the three sections were collected at the beginning, middle and end
338 of the time starting when the other goat in the pair received food, until this goat finished
339 eating. This ensured that the data were collected while the subject could see and hear
340 the paired goat eating. The subject was thus likely to be experiencing a negative state of
341 frustration throughout data collection. By contrast, the situation in which both goats were
342 not eating (after the pair goat finished) or the situation in which the subject was itself
343 eating are likely to trigger different arousal and valence and were thus not considered for
344 analyses. Finally for the Isolation situation, the three sections were collected at the
345 beginning, middle and end of the time starting 1 min after the subject was placed in the
346 isolation pen, until the end of the Isolation situation 4 min later (isolation duration = 5
347 min). This allowed the goat to settle in the isolation pen and to be likely to be
348 experiencing a negative state of isolation, before we started data collection.

349 From the ECG trace and breathing wave, we analysed the following parameters:
350 heart rate, heart-rate variability (root mean square of successive R-R interval difference,

351 "RMSSD") and respiration rate. The heart-rate (beats/min) and respiration-rate
352 (breaths/s) averages were obtained automatically from the software. Individual inter-
353 heart-beat (RR) intervals (ms) were also extracted, in order to calculate RMSSD (ms).

354

355 *Behavioural measures*

356 For a similar reason than for the physiological parameters (i.e. to measure parameters
357 during well-defined positive/negative emotional state), for the Feeding situation, we
358 analysed the behaviour of the goats during the two events when the experimenter came
359 back from outside the stable complex towards the goats with food (i.e. when the goats
360 could see the experimenter bringing them food; mean total time scored per goat \pm SD =
361 29.55 ± 5.54 s). For the Frustration situation, we analysed the behaviour during the time
362 starting when the other goat in the pair received food, until this goat finished eating (i.e.
363 when the goats could see or hear the other goat eating; mean total time scored per goat
364 \pm SD = 176.50 ± 35.91 s). For the Isolation and Control situation, we analysed the
365 behaviour during the last 4 min of the test (i.e. 1 min after the subject was placed in the
366 test pen; time scored per goat per test = 240 s).

367 We scored behavioural parameters that could potentially be affected by emotions
368 (Boissy et al., 2011; Reefmann, Bütikofer Kaszàs, et al., 2009a; Reefmann, Wechsler, et
369 al., 2009). The following parameters were scored using CowLog 1.1 (open source
370 software for coding behaviours from digital videos; Hänninen & Pastell, 2009); the time
371 spent with the tail raised (i.e. tail raised above the perpendicular to the backbone), the
372 duration of locomotion (defined as at least two legs moving), the number of rapid head
373 movements (i.e. < 1 s in duration), and the number of calls produced. We also scored
374 the time spent with the ears orientated forward (tip of the ear pointing forward),
375 backwards (tip of the ear pointing backward), horizontal (perpendicular to the headrump
376 axis), or asymmetrical (right and left ears in different positions, such as one pointing
377 forward and the other one horizontal or backwards; Boissy et al., 2011; Reefmann,
378 Bütikofer Kaszàs, et al., 2009a).

379 We calculated the rate of occurrence (per minute) for the number of rapid head
380 movements and for the number of calls, and the proportion of the total time spent
381 performing the behaviour, for the other behaviours (Table 2). Therefore, the difference in
382 duration between the various situations was taken into consideration. When the head,
383 ears or tail were not easily observable because of the position of the camera or goat in
384 the pen, the proportion of behaviour was calculated over the time during which we were
385 able to score the behaviour accurately, instead of the total time. On two occasions, it was
386 not possible to score the position of the ears and the head movements (one goat during
387 the Feeding situation) or the position of the tail (one goat during the Feeding situation).
388 Therefore, sample sizes differ slightly between parameters (see sample sizes in Table 3).

389

390 *Vocal measures*

391 Vocalisations were imported into a computer at a sampling rate of 44.1 kHz and saved in
392 WAV format at 16-bit amplitude resolution. We used Praat v.5.3.41 DSP Package
393 (Boersma & Weenink, 2009) and Seewave (Sueur, Aubin, & Simonis, 2008) for
394 subsequent analyses. Calls were visualised on spectrograms in Praat (FFT method,
395 window length = 0.03 s, time steps = 1000, frequency steps = 250, Gaussian window
396 shape, dynamic range = 60 dB). We selected all good quality calls recorded during each
397 situation (total = 180 calls; 40 for Feeding, 80 for Frustration and 60 for Isolation; 8.18
398 ± 7.76 calls per goat; range = 0 (3 goats) – 30; Table 1). Because calls were produced
399 intermittently by the goats (unlike physiological and behavioural data that could be
400 acquired continuously), we used a more opportunistic approach in order to obtain
401 adequate sample sizes of vocalisations. During the Feeding situation, we analysed calls
402 produced between the time when the experimenter approached the goats for the first
403 time with the food, until they were rewarded. During the Frustration situation, we
404 analysed calls produced between the time when the other goat received food and the
405 time preceding the return of the experimenter toward the subject to give it food. During
406 the Isolation situation, we analysed all the calls produced while the subject was in the
407 isolation pen. Calls were never produced in bouts. Because consecutive calls produced in

408 bouts are more likely to be homogeneous, we therefore avoided pseudoreplication (Reby,
409 Cargnelutti, & Hewison, 1999).

410 According to the source-filter theory of voice production (Fant, 1960), mammal
411 vocalisations are generated by vibrations of the vocal folds (source, determining the
412 fundamental frequency, "F0"), and are subsequently filtered by the supralaryngeal vocal
413 tract (filter, producing amplified frequencies called "formants", Titze, 1994; Taylor and
414 Reby, 2010). We extracted source- and filter-related vocal parameters as well as
415 intensity and duration measures using a custom built program in Praat. This program
416 batch processed the analyses and exporting of output data (Charlton, Zhihe, & Snyder,
417 2009a; Reby & McComb, 2003). The vocal parameters measured are listed in Table 2 and
418 the analyses are detailed in the Appendix.

419 The settings for the analyses were adapted from Briefer & McElligott (2011a).
420 Goat contact calls vary between individuals, particularly at the level of F0-related
421 parameters, formant-related parameters and energy quartiles (see Table 2 for
422 definitions; Briefer & McElligott, 2011a). Therefore, the most appropriate settings to
423 accurately detect F0 (i.e. pitch floor and pitch ceiling) and formants (i.e. maximum
424 number of formants and maximum formant value; see Appendix for details) with Praat
425 differed between individuals. In order to prevent biases linked to the settings used for
426 the analyses, the same settings were used for all calls (i.e. produced during all
427 situations) of a given individual. We included 13 vocal parameters in our analyses. Some
428 parameters (formants) could not be measured in every call, resulting in a small
429 proportion of missing values. Therefore, the sample size (number of calls) differs
430 between the vocal parameters (see sample size in Table 5).

431

432 *Statistical Analysis*

433

434 First, in order to investigate which physiological, behavioural or vocal parameters would
435 be more useful as indicators of emotions (as opposed to which ones were correlated and
436 therefore redundant), we tested for potential correlations between the mean parameter

437 values for each individual using Spearman's rank correlation. Then, to test for differences
438 in heart rate between situations and determine arousal levels, we carried out a linear
439 mixed effects model (LMM; lmer function, lme4 library; Pinheiro & Bates, 2000) in R
440 3.0.2 (R Development Core Team, 2013). This model included heart rate as a response
441 variable, and the sex and age of the goats as fixed factors to control for their effects. The
442 situation (Control, Feeding, Frustration and Isolation) was included as a fixed factor.
443 Finally, the breed, and identity of the goats nested within the test pair were included as
444 crossed random factors, in order to control for repeated measurements of the same
445 subjects, for the impact of the goats within a pair on each other, and for breed
446 differences (Table 1). Then, two-by-two comparisons between the four emotional
447 situations were carried out using LMMs including the same control, fixed and random
448 factors. We applied a Bonferroni correction at $\alpha = 0.017$ ($0.05/3$ comparisons) for these
449 *post-hoc* tests. Based on these results, we ranked the situations according to the heart
450 rate values they triggered; we attributed the highest arousal level to the situation
451 triggering the highest heart rate and the lowest arousal level to the one triggering the
452 lowest heart rate. Situations that did not differ in heart rate were considered to be of the
453 same arousal level.

454 We ran further models to test the effects of arousal and valence on the other
455 physiological, behavioural and vocal parameters measured (Table 2). Separate LMMs
456 were set up for each parameter. All of these models included the parameter as a
457 response variable and the same control and random factors as listed above for heart rate
458 (sex and age of the goats as fixed factors; breed, and identity of the goats nested within
459 the test pair, as crossed random factors). The proportion of time spent moving
460 (Locomotion) was also included as a fixed factor for the physiological parameters, to
461 control for its effect. The extent of mouth opening influences the resonant properties of
462 the vocal tract (Titze, 1994). Several vocal parameters thus differ between closed- and
463 open-mouth calls (Sèbe, Duboscq, Aubin, Ligout, & Poindron, 2010). For this reason, we
464 added the type of call (open-mouth, closed-mouth or mixed, referring to calls containing
465 both open-mouth and closed-mouth parts) as a fixed factor in the models carried out on

466 the vocal parameters to control for the potential differences between call types (see
467 Appendix Table A1 for the results of the control factors).

468 Because arousal levels and valence were correlated (Spearman's rank correlation:
469 $r = 0.15$, $p = 0.006$), they were not included as factors in the same models. Instead, we
470 first ran one set of models with arousal level (1-2; see Results, *Determination of Arousal*
471 *Levels using Heart Rate*) as a fixed effect and another set with valence (Control situation
472 = 0; Feeding = +1; Frustration and Isolation situations = -1) as a fixed effect. Then, for
473 each parameter that was significantly affected by both arousal and valence, we used a
474 model selection procedure based on the Akaike's information criterion adjusted for small
475 sample size (AIC_c) to identify which of arousal or valence best explained the parameter
476 (Burnham & Anderson, 2002). We used AIC_c instead of AIC, because AIC_c converges to
477 AIC as sample size increases and should be used by default (Symonds & Moussalli,
478 2011). When the difference between the AIC_c values of two models (ΔAIC_c) is less than 2
479 units, both models have support and can be considered competitive. Models with ΔAIC_c
480 ranging from 3 to 7 have considerably less support by the data, and models with $\Delta AIC_c >$
481 10 are poorly supported. Akaike weights (ω_i) indicate the probability that a particular
482 model has more or less support from the data among those included in the set of
483 candidate models (Burnham & Anderson, 2002; Burnham, Anderson, & Huyvaert, 2011).

484 To test for further differences between the Frustration and Isolation situations,
485 which were both assumed to be of negative valence, we carried out further LMMs. We
486 included in these models (one for each parameter) the same control and random factors
487 as mentioned above (sex and age of the goats, Locomotion (physiology only) and call
488 type (vocalisations only) as fixed factors; breed, and identity of the goats nested within
489 the test pair as crossed random factors, and the situation (Frustration and Isolation) as a
490 fixed factor (see Appendix Table A1 for these results)).

491 The residuals were checked graphically for normal distributions and
492 homoscedasticity. To satisfy assumptions, we used log transformations for RMSSD,
493 ResRate, F0mean, F0end, F0range, FMextent, AMextend, Q50%, Q75% (see Table 2 for
494 abbreviations). Some of the behavioural parameters measured in proportions were logit-

495 transformed (EarsForward, HeadMov, and Locomotion). These log- and logit-transformed
496 physiological, behavioural and vocal parameters were then entered into models fitted
497 with Gaussian family distribution and identity link function. None of the other behavioural
498 parameters (see Table 2) met statistical assumptions despite logit transformation. They
499 were thus transformed to binomial data (behaviour occurs = 1; does not occur = 0), and
500 entered into generalised linear mixed models, fitted with binomial family distribution and
501 logit link function (glmer function, lme4 library). For each model, we assessed the
502 statistical significance of the factors by comparing the model with and without the factor
503 included using likelihood-ratio tests (LRT). To this aim, and in order to compare models
504 with AIC_c , all models were fitted with maximum likelihood estimation. The significance
505 level was set at $\alpha = 0.05$. All means are given with standard deviations.

506

507 *Ethics*

508

509 Animal care and all experimental procedures were in accordance with the ASAB/ABS
510 Guidelines for the Use of Animals in Research (Guidelines for the Use of Animals, 2014).
511 The research plan was reviewed by the UK Government Home Office inspector for Queen
512 Mary, University of London. All measurements were non-invasive, and the negative
513 situations (Frustration and Isolation) lasted not more than 5 min each. The stress levels
514 of the goats were monitored throughout the tests (ECG trace and breathing wave were
515 transmitted and displayed, in real time on the laptop) to ensure that subjects did not
516 become overly stressed, in which case the test would be halted and the subject would
517 not be tested anymore. However, this did not happen and none of the goats had to be
518 removed from the experiments.

519

520 RESULTS

521

522 *Determination of Arousal Levels using Heart Rate*

523

524 The analysis of heart rate as a function of the emotional situations revealed two arousal
525 levels (1 and 2; Fig. 1). Heart rate differed according to the situation (LMM: $X^2 = 53.03$,
526 $df = 3$, $p < 0.0001$), with the lowest value occurring during the Control situation. These
527 heart rates were not different from those obtained for the Isolation situation (Control vs
528 Isolation; LMM: $X^2 = 0.68$, $df = 1$, $p = 0.41$). Therefore, we attributed an arousal level of
529 1 (lowest) to the Control and Isolation situations. The heart rates measured during the
530 Isolation situation were significantly lower than those measured during the Frustration
531 situation (Isolation vs Frustration; LMM: $X^2 = 12.24$, $df = 1$, $p = 0.0005$). Finally, the
532 rates for the Frustration situation were not significantly different from those obtained for
533 the Feeding situation, after Bonferroni correction (Frustration vs Feeding; LMM: $X^2 =$
534 4.36 , $df = 1$, $p = 0.037$; Bonferroni, $\alpha = 0.017$). Frustration and Feeding situations thus
535 received an arousal level of 2. To summarize, the arousal levels based on heart rate were
536 1 for the Control and Isolation situations (mean \pm SD = 108.75 ± 15.38 beats/min) and
537 2 for the Frustration and Feeding situations (mean \pm SD = 123.00 ± 23.52 beats/min).

538

539 *Physiological Responses*

540

541 Correlation analyses between the mean physiological parameters of each goat showed no
542 associations between RMSSD and RespRate (Spearman's rank correlation: $r = -0.09$, $n =$
543 22 goats, $p = 0.69$). The models investigating the link between physiological parameters
544 and emotional arousal and valence of the situations revealed that the two measured
545 parameters (RMSSD and RespRate) were influenced by arousal and not by valence (Table
546 3; Appendix Table A2). RMSSD decreased and RespRate increased with arousal (Table 3).
547 To summarise, RMSSD and RespRate were good indicators of arousal, as they were
548 clearly more affected by arousal levels than valence. During higher arousal situations,

549 goats had lower heart-rate variability (RMSSD) and higher respiration rates. There were
550 no clear physiological indicators of valence.

551

552 *Behavioural Responses*

553

554 Correlation analyses between the mean behavioural parameters of each goat showed
555 several associations between most of the ear positions (EarsForw, EarsHoriz and
556 EarsAsym) and some of the other behavioural parameters (HeadMov, Locomotion and
557 TailUp; Table 2). EarsBack was only correlated with other ear position parameters
558 (EarsForw and EarsAsym). Finally, the number of calls per minute (Calls) was not
559 correlated with any of the other behavioural parameters.

560 The analyses of behavioural parameters in relation to the emotional arousal and
561 valence of the situations revealed that all measured parameters were affected by arousal
562 (Table 3; Appendix Table A2). HeadMov, Locomotion, EarsForw, Calls and TailUp
563 increased with arousal, while EarsHoriz, EarsBack and EarsAsym decreased. Four of the
564 parameters were also affected by valence (HeadMov, EarsBack, EarsAsym and TailUp;
565 Table 3; Appendix Table A2). EarsBack decreased and TailUp increased from negative to
566 positive valence. HeadMov and EarsAsym were not affected consistently by valence
567 (level: HeadMov, - > 0 < +; EarsAsym, - < 0 > +). AIC_C comparison revealed that the
568 variation in HeadMov was better explained by arousal (100% chance to be the best
569 model) than valence, while variation in EarsBack and EarsAsym was better explained by
570 valence (100% and 99% chance respectively to be the best model) than arousal. For
571 TailUp, the ΔAIC_C was 5.13, indicating that the model including valence had considerably
572 more support by the data than the model including arousal. The model including valence
573 had 93% chance to be the best model (chance level = 50%; Table 4).

574 To summarise, HeadMov, Locomotion, EarsForw, EarsHoriz and Calls were good
575 indicators of arousal, as they were clearly more affected by arousal levels than valence.
576 During high arousal situations, goats displayed more head movements, moved more, had
577 their ears pointed more often forward and less often to the side (horizontal) and

578 produced more calls. By contrast, EarsBack and TailUp were good indicators of valence,
579 as they changed consistently from negative to positive valence (unlike EarsAsym), and
580 were more affected by valence levels than arousal ($\Delta AIC_c > 5$). In positive situations, as
581 opposed to negative ones, goats had their ears orientated backward less often and spent
582 more time with the tail up.

583

584 *Vocal Responses*

585

586 Correlation analyses between the mean vocal parameters of each goat showed several
587 associations between most of the F0-related parameters (F0mean, F0end, F0range and
588 FMextent; Table 2). Some of these F0-related parameters (F0mean and F0end) were also
589 correlated with energy quartiles (Q25%, Q50%, and Q75%), which were all correlated to
590 each other. AMextent was only correlated with F0mean. F1mean was correlated with
591 F2mean. Finally, F2mean, F3mean and F4mean were all related to each other.

592 Our analyses of vocal parameters as a function of the arousal levels and valence
593 of the situations revealed six parameters that were influence by arousal (Table 5;
594 Appendix Table A2). F0mean and F0end (which characterise F0 contour over time), as
595 well as Q25%, Q50%, and Q75% increased with arousal, while F1mean decreased.
596 F4Mean also tended to increase, but the effect of arousal on this parameter was only
597 marginally significant ($p = 0.055$). Two parameters were influence by valence (Table 5;
598 Appendix Table A2). F0range and FMextent (which characterise F0 variation within the
599 call), all decreased from negative to positive valence. The other parameters were neither
600 affected by arousal, nor by valence (Table 5).

601 To summarize, F0mean, F0End, Q25%, Q50%, Q75% and F1Mean were good
602 indicators of arousal, as they were clearly more affected by arousal than valence. With an
603 increase in arousal, goats produced calls with higher F0 and higher energy distribution
604 (i.e. energy quartiles). Furthermore, the first formant frequency decreased, while the
605 fourth formant tended to increase. By contrast, F0range and FMextent were good
606 indicators of valence, as they were clearly more affected by valence than arousal. In

607 positive situations, as opposed to negative ones, goats produced calls with a lower
608 fundamental frequency range and smaller frequency modulations (i.e. calls with less
609 frequency variations; Fig. 2 and Audio A1).

610

611 **DISCUSSION**

612

613 We investigated changes in physiological, behavioural and vocal parameters between
614 situations potentially triggering different arousal and valence, in order to identify non-
615 invasive indicators of emotions in goats. We found physiological, behavioural and vocal
616 indicators of arousal. All these parameters changed consistently with increasing arousal
617 and were clearly more affected by arousal than valence. We also found behavioural and
618 vocal indicators of valence that changed consistently from negative to positive valence,
619 and were clearly more affected by valence than arousal. Arousal indicators could help to
620 identify and therefore minimize stress during negative situations. By contrast, valence
621 indicators could help to differentiate between negative and positive situations, in order to
622 reduce negative emotions and increase positive ones. In particular, the behavioural
623 indicators that we found are relatively easy to observe. The potential to more effectively
624 monitor animal emotions (and therefore also moods) is critical to our overall
625 understanding of animal behaviour and wellbeing in general (Boissy et al., 2007; Mendl
626 et al., 2010; Nettle & Bateson, 2012), as well as our understanding of the evolution of
627 emotions.

628

629 *Physiological Indicators*

630

631 We used heart rates in this study to determine arousal levels triggered by the various
632 situations. We found that this parameter was higher during the food frustration and
633 anticipation of food reward situations than during the isolation and control situations.
634 Isolation in social species normally induces high stress levels and an increase in heart
635 rates (e.g. in ungulates; cattle, *Bos taurus*, Boissy & Le Neindre, 1997; sheep,
636 Reefmann, Wechsler, et al., 2009; goats, Aschwanden, Gygax, Wechsler, & Keil, 2008).
637 Therefore, it is surprising that our social isolation situation induced heart-rate values that
638 were similar to the control situation. Our tests were preceded by three days of
639 habituation, in order to minimize stress linked to novelty. Goats seemed to decrease

640 stress-related behaviours over the days of habituation (EFB, personal observation),
641 suggesting that they could have fully habituated to the isolation situation after three
642 days. This differs from Siebert et al. (2011), which did not find clear evidence for
643 habituation to repeated isolation sessions in the behavioural and vocal responses of
644 goats. This could be due to the much longer isolation sessions used by Siebert et al.
645 (2011; 30 min) compared to ours (5 min).

646 The physiological measures that we collected (heart rate, heart-rate variability
647 and respiration rate) are involved in the sympathomedullary pathway axis (SAM) stress
648 response, which prepares the animal to react to a stressor (Cannon, 1929; Seyle 1976).
649 Stress has been defined as a nonspecific response of the body to any demand made upon
650 it, whether positive or negative (Seyle, 1976). An increase in both negative and positive
651 arousal is thus normally accompanied by (among other parameter changes), an increase
652 in heart rate (used in our study to determine the levels of arousal triggered by the
653 various situations), and an increase in respiration rate (von Holst, 1998). Accordingly, we
654 found that respiration rate increased with increasing arousal (heart rate), independently
655 of the valence of the situation.

656 Heart rate represents the interaction between sympathetic (increases heart rate)
657 and vagal (reduces heart rate) regulation. By contrast, heart rate variability mainly
658 depends on vagal influences and thus indicates when the vagal branch of the autonomic
659 nervous system is activated (von Borell et al., 2007). This parameter increases (i.e. more
660 variable heart rate due to an increase in successive R-R interval difference) when the
661 parasympathetic system is activated. In our study, heart rate variability (RMSSD; root
662 mean square of successive R-R interval difference) increased with decreasing arousal
663 levels (heart rate), indicating that the parasympathetic system was activated during our
664 low arousal situations. According to our criterion, RMSSD was thus also an accurate
665 indicator of arousal. Our two physiological parameters (RespRate and RMSSD) were not
666 correlated and therefore are both good, independent indicators of arousal.

667 We did not find any good physiological indicator of valence. High vagal tone and
668 vagal activation have been suggested to indicate efficient autonomic regulatory activity

669 and be associated with positive emotions, thus implying that RMSSD could be a good
670 indicator of valence, notably in pigs (Zebunke, Langbein, Manteuffel, & Puppe, 2011) and
671 sheep (Reefmann, Wechsler, et al., 2009). However, these studies did not control for the
672 effect of arousal, as they compared situations of opposite valence, but also of different
673 arousal (e.g. grooming as positive versus isolation as negative). In our study, RMSSD
674 was not influenced by valence, despite controlling for the degree of movement
675 (Locomotion). Similarly, Gygax et al. (2013) did not find any difference in RMSSD
676 between two situations of different valence and likely similar arousal in goats. We
677 suggest that heart rate variability, in a similar manner to most physiological parameters,
678 is affected by arousal more than valence and thus constitutes an indicator of arousal.

679

680 *Behavioural Indicators*

681

682 The stress response prepares an animal to be more alert and vigilant, and to behave
683 appropriately when faced with a stressor (e.g. Cannon, 1929). When the stimulus
684 triggering the change in arousal enhances fitness (e.g. food reward), the animal should
685 approach it, whereas when the stimulus threatens fitness (e.g. predator), the animal
686 should avoid it (e.g. flight; Mendl et al., 2010). Accordingly, in our study, goats displayed
687 more head movements and moved more during high arousal than low arousal situations,
688 independently of the valence. Similarly, in Siebert et al. (2011), goat locomotion
689 increased with arousal between complete isolation and partial isolation (supposedly
690 higher arousal than complete isolation, because of the permanent sensory feedback from
691 adjacent pen mates). In our study, goats also had their ears pointed forward more often
692 and less often to the side (horizontal), which could indicate vigilance. Call rate generally
693 increases with arousal in most species (Briefer, 2012). Accordingly, our results showed
694 an increase in the number of calls per minute (Calls) with arousal, independently of
695 valence (i.e. in both negative and positive situations).

696 We identified two promising behavioural indicators of valence. Goats had their
697 ears orientated backward less often and spent more time with the tail up in positive

698 situations compared to negative ones. Ear and tail positions were also suggested as
699 indicators of emotions notably in sheep (Boissy et al., 2011; Reefmann, Bütikofer Kaszàs,
700 et al., 2009a; Reefmann, Wechsler, et al., 2009), and pigs (Reimert, Bolhuis, Kemp, &
701 Rodenburg, 2013). Reefmann, Bütikofer Kaszàs, et al. (2009) and Reefmann, Wechsler,
702 et al. (2009a) showed that horizontal ears are associated with positive situations in
703 sheep. However, in these studies, some of the positive situations (e.g. feeding on fresh
704 hay or grooming) and negative situations (e.g. separation from group members) might
705 have differed also in arousal levels. In a similar manner to our study, pigs in negative
706 situations (anticipation of aversive situation) had their tails more often in a low position
707 and ears more often backward than positive situations (anticipation of a rewarding
708 situation; Reimert et al., 2013). By contrast, sheep tails were held up during separation
709 from group members (negative), but not during rumination (intermediate) or when
710 feeding on fresh hay (positive; Reefmann, Bütikofer Kaszàs, et al., 2009a). Alternative
711 techniques for measuring ear and tail movements (e.g. non-invasive electromyography
712 measuring ear and tail muscle activity) could help to obtain more precise results and
713 allow accurate cross-species comparisons. Because ear position in our study was
714 correlated with other behavioural parameters and clearly indicated both arousal
715 (EarsForward and EarsHorizontal) and valence (EarsBackward), we suggest that the
716 observation of ear positions is a promising tool to assess emotions.

717

718 *Vocal Indicators*

719

720 We found that parameters describing F0-contour (source-related parameters; F0mean,
721 and F0End) increased with arousal levels. This could have resulted from an increase in
722 the tension of the vocal folds after contraction of the cricothyroid muscles, or from
723 stronger sub-glottal air pressure (Fant, 1960; Titze, 1994). We also found that higher
724 arousal levels were characterized by higher energy quartiles (Q25%, Q50%, Q75%).
725 Filter-related parameters (i.e. formants and the energy distribution) mostly depend on
726 the shape and length of the vocal tract, and can be modified by laryngeal retraction (e.g.

727 goats, dogs *Canis familiaris*, pigs and cotton-top tamarins *Sagunius oedipus*; Fitch, 2000;
728 fallow deer *Dama dama*; McElligott et al., 2006). Mammals could also potentially
729 constrict their pharynx (i.e. increase the tension of vocal tract walls), which results in a
730 shift in energy distribution towards higher frequencies, but this phenomenon has, until
731 now, only been studied in humans (Scherer, 1986) and birds (Riede, Beckers, Blevins, &
732 Suthers, 2004). The shift in the energy distribution towards higher frequencies that we
733 observed could thus be caused by a less pronounced retraction of the larynx or/and an
734 increase in pharyngeal constriction with an increase in arousal. Although the energy
735 distribution mainly depends on the filter process, our correlations between vocal
736 parameters showed that this parameter was correlated with F0 (and therefore with its
737 harmonics). As a result, the increase in energy quartiles with arousal could also have
738 been a side effect of the increase in F0. Similar increases in F0 and energy quartiles with
739 arousal are commonly found in humans (Scherer, 2003), other mammals (Altenmüller,
740 Schmidt, & Zimmermann, 2013; Briefer, 2012; Lingle, Wyman, Kotrba, Teichroeb, &
741 Romanow, 2012), and even birds (zebra finch, *Taeniopygia guttata*, Perez et al., 2012),
742 suggesting highly conserved vocal indicators of arousal throughout evolution.

743 Surprisingly, the increase in energy quartiles with arousal was only accompanied
744 by a marginally significant increase in the fourth formant (F4; $p = 0.055$). By contrast,
745 the first formant (F1) decreased. These discrepancies were confirmed by a lack of
746 correlation between energy quartiles and formants. Higher formants (e.g. F3, F3) depend
747 mainly on the length of the vocal tract (Fant, 1960; Fitch & Hauser, 1995), while lower
748 formants (e.g. F1, F2) depend more on the shape of the vocal tract. Our results could be
749 explained by a less pronounced retraction of the larynx, which shortens the vocal tract
750 and induces a higher F4, with an increase in arousal (Fant, 1960; Titze, 1994). The
751 decrease in F1 might require more subtle changes in the configuration of the pharyngeal
752 regions and oral and nasal cavities, and opening of the mouth. Indeed, mouth
753 opening/closing and lip protrusion/retraction or lip rounding/spreading can also be used
754 to modify formant frequencies, at least in primates (Hauser, Evans, & Marler, 1993;
755 Hauser & Ybarra, 1994; Riede, Bronson, Hatzikirou, & Zuberbühler, 2005). However, this

756 suggestion requires further analysis of goat behaviour while vocalizing (Riede et al.,
757 2005). Several of our vocal parameters indicating arousal (F0mean, F0End, Q25-Q75%
758 and F1Mean) were correlated with each other. Energy quartiles (Q25-Q75%) are easier
759 to measure than F0 or formants and could constitute ideal indicators of arousal in goats
760 and maybe other ungulates as well.

761 We found that during positive situations, goats produced calls with lower
762 fundamental frequency range (F0range), as well as smaller frequency modulations
763 (FMextent) than during negative situations. The fundamental frequency thus presented
764 less variation during positive than negative emotions. These measures characterising F0
765 variation (F0Range and FMextent) were correlated. F0Range was more clearly affected by
766 valence than FMextent. This parameter, which is also easier to measure than FMextent,
767 could thus be selected as a clear valence indicator in goats. A decrease in F0range from
768 negative to positive situations has also been observed in humans (Hammerschmidt &
769 Jürgens, 2007), and elephants (*Loxodonta africana*, Soltis et al., 2011). Similarly, lower
770 variation in F0 (cumulative variation of F0) in positive than in negative situation has been
771 found in dogs (Taylor, Reby, & McComb, 2009). During positive emotions, it thus seems
772 that vocal folds vibrate at a more stable rate than in negative emotions, resulting in more
773 stable F0 over time. However, very few studies have been carried out on vocal indicators
774 of valence and it is thus difficult to make general conclusions on the evolution of vocal
775 correlates of valence.

776

777 *Conclusion*

778 By merging recent frameworks developed to measure animal vocalisations (source-filter
779 theory; Taylor and Reby 2010) and emotions (Mendl et al., 2010), we have identified
780 several non-invasive, promising indicators of arousal and valence. These physiological,
781 behavioural and vocal indicators could be very useful to differentiate situations eliciting
782 negative emotions from those eliciting positive ones, in order to promote the
783 implementation of positive animal states (Boissy et al., 2007). Further experiments
784 validating these indicators using different emotional situations (e.g. partial versus total

785 isolation; Siebert et al., 2011), could allow us to determine the extent to which these
786 indicators can be used across contexts. We believe that our approach, which
787 simultaneously measures the effects of emotional arousal and valence, could lead to
788 more accurate monitoring of animal emotions and a more comprehensive understanding
789 of the evolution of emotions.

790

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792

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800

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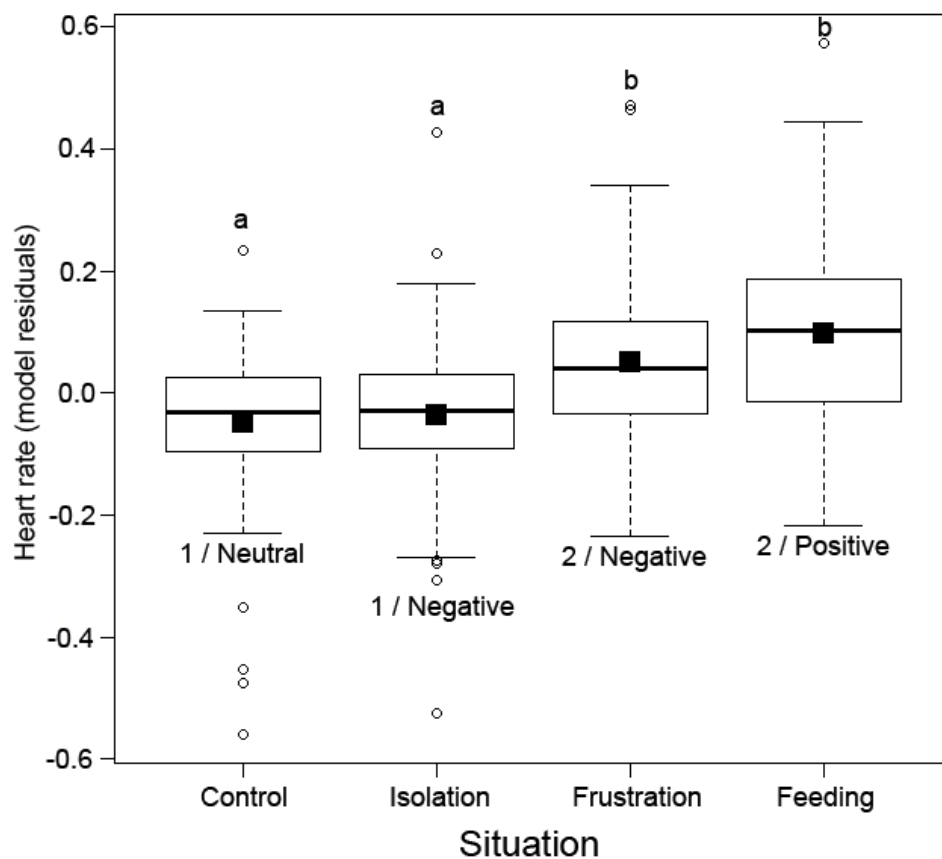
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- 1006



1008

1009 **Figure 1.** Heart rate as a function of emotional situations. Model residuals of heart rate

1010 controlled for sex and age of the goats, individual identity, test pair and breed for each of

1011 the experimental situations (Control, Feeding, Frustration and Isolation situations; box-

1012 and-whiskers plot, the horizontal line shows the median, the box extends from the lower

1013 to the upper quartile, and the whiskers to the most extreme data point). The black

1014 squares indicate the mean. Same letters (a, b) indicate that situations did not

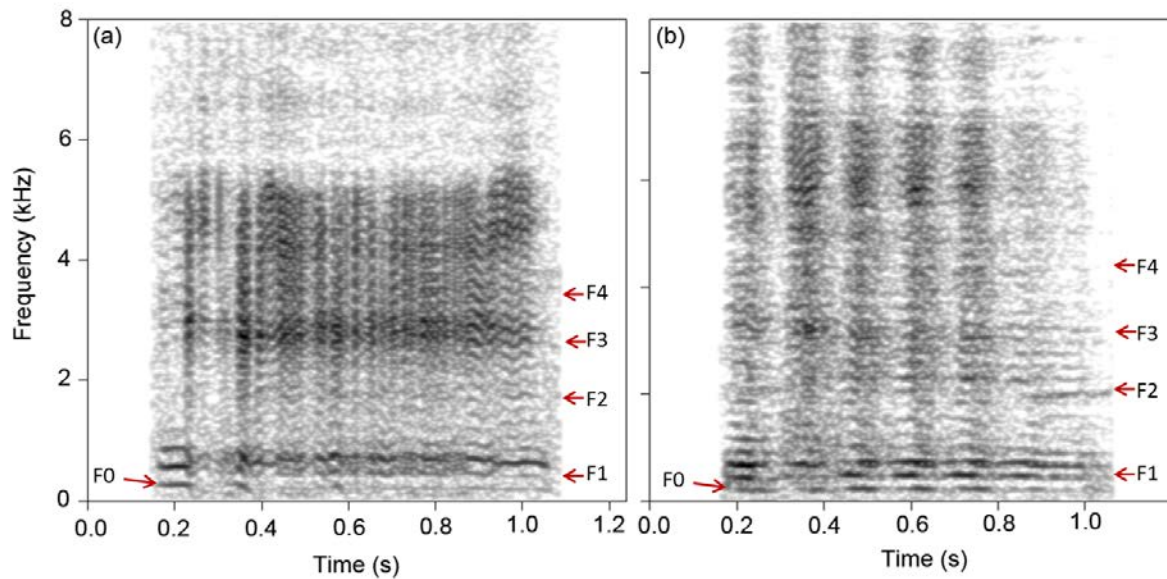
1015 significantly differ. Based on these results, situations marked with an (a) received an

1016 arousal level of 1, situations marked with a (b) received an arousal level of 2. Arousal

1017 levels (1 or 2) and valence (Neutral, Positive and Negative) corresponding to the

1018 situations are also indicated under the box-and-whiskers plot (arousal / valence).

1019



1020

1021

1022 **Figure 2.** Spectrograms of negative and positive calls. (a) call produced during the
 1023 negative situation; (b) call produced during the positive situations by the same goat.

1024 Positive calls have a lower fundamental frequency (F0) range and smaller frequency
 1025 modulations than negative calls. The first four formants are also indicated on the right of
 1026 the spectrogram. These calls are available as audio files (Audio A1).

1027

1028 **Appendix**

1029 *Acoustic Analysis*

1030

1031 Here, we provide a detailed description of the acoustic analysis. The source-related
1032 acoustic features (fundamental frequency, F0), filter-related acoustic features (formants
1033 and energy quartiles), and intensity features that we measured (13 parameters) are
1034 detailed below (Praat commands are indicated in brackets).

1035 Source-related acoustic features were measured by extracting the F0 contour of
1036 each call using a cross-correlation method ([Sound: To Pitch (cc) command], time step =
1037 0.01 s, pitch floor =100-150 Hz, pitch ceiling = 300-600 Hz). For each extracted F0
1038 contour, we measured the following vocal parameters: the frequency value of F0 at the
1039 end (F0end) of the call, the mean F0 frequency values across the call (F0mean), and the
1040 F0 frequency range (F0range). To characterize F0 variation along the call, we measured
1041 the mean peak-to-peak variation of each F0 modulation (FMExtent, Charlton et al.,
1042 2009a; Charlton, Zhihe, & Snyder, 2009b).

1043 Filter-related (formants) acoustic features were measured by extracting the
1044 contour of the first four formants of each call using Linear Predictive Coding analysis
1045 (LPC; [Sound: To Formant (burg) command]: time step = 0.01 s, maximum number of
1046 formants = 4–5, maximum formant = 3000–5500 Hz, window length = 0.05 s). To check
1047 if the Praat software accurately tracked the formants, the outputs of the LPC analysis
1048 were visually inspected together with the spectrograms. Spurious values were deleted
1049 and we corrected for octave jumps when necessary. For each call we collected the mean
1050 (F1-4mean) values of the formants. Finally, we measured the frequency values at the
1051 upper limit of the first (Q25%), second (Q50%) and third (Q75%) quartiles of energy,
1052 using a linear amplitude spectrum applied to the whole call.

1053 We measured intensity characteristics by extracting the intensity contour of each
1054 call [Sound: To Intensity command]. We then included the mean peak-to-peak variation

1055 of each amplitude modulation in our analyses (AMextent; see Charlton et al. 2009b for
1056 details of these parameters). We also included the total duration of each call (Dur).
1057

1058 **Tables**

1059 **Table 1.** Characteristics of the goats used: sex, age and breed; as well as the number of
 1060 calls analyzed for each goat.

1061

Goat	Sex	Age (years)	Breed	Number of calls
1	Female	11	Anglo-Nubian	8
2	Female	16	British Toggenburg	0
3	Female	8	Pygmy Goat	6
4	Female	11	Golden Guernsey	12
5	Female	7	British Alpine	2
6	Female	5	British Alpine	8
7	Female	14	British Toggenburg	10
8	Female	7	British Saanen	19
9	Female	7	British Toggenburg	3
10	Female	13	British Toggenburg	20
11	Female	17	British Saanen	0
12	Male	11	Pygmy Goat	5
13	Male	10	Golden Guernsey	17
14	Male	8	Pygmy Goat	0
15	Male	10	British Toggenburg	2
16	Male	7	British Toggenburg	11
17	Male	9	British Saanen	8
18	Male	4	Boer	12
19	Male	7	British Toggenburg	10
20	Male	3	Boer	9
21	Male	9	Mixed Breed	6
22	Male	6	Golden Guernsey	2

1062

1063 **Table 2.** Abbreviations, definition and correlations for the physiological, behavioural and
 1064 vocal parameters.
 1065

	Abbreviation	Correlated with	Parameter
Physiology	RMSSD (ms)	HR	Root mean square successive difference
	RespRate (breaths/s)	HR	Respiration rate
Behaviour	HeadMov (min ⁻¹)	EarsHoriz	Number of rapid head movement per minute
	Locomotion	EarsForw, EarsAsym, TailUp	Proportion of time spent moving
	EarsForw	Locomotion, EarsHoriz, EarsBack, EarsAsym, TailUp	Proportion of time spent with the ears orientated forward
	EarsHoriz	HeadMov, EarsForw, TailUp	Proportion of time spent with the ears orientated horizontally
	EarsBack	EarsForw, EarsAsym	Proportion of time spent with the ears orientated backward
	EarsAsym	Locomotion, EarsForw, EarsBack	Proportion of time spent with the ears asymmetrical (different orientation for the right and left ears)
	TailUp	Locomotion, EarsForw, EarsHoriz	Proportion of time spent with the tail up
	Calls (min ⁻¹)	None	Number of calls per minute
Vocalisations	Dur (s)	None	Duration of the call
	F0mean (Hz)	F0end, F0range, FMextent, AMextent, Q25%, Q50%	Mean F0 frequency value across the call
	F0end (Hz)	F0mean, F0range, Q50%, Q75%	Frequency value of F0 at the end of the call
	F0range (Hz)	F0mean, F0end, FMextent	Difference between F0Max and F0Min
	FMextent (Hz)	F0mean, F0range	Mean peak-to-peak variation of each F0 modulation
	AMextent (dB)	F0mean	Mean peak-to-peak variation of each amplitude modulation
	Q25% (Hz)	F0mean, Q50%, Q75%	Frequency value at the upper limit of the first quartiles of energy
	Q50% (Hz)	F0mean, F0end, Q25%, Q75%	Frequency value at the upper limit of the second quartiles of energy
	Q75% (Hz)	F0end, Q25%, Q50%	Frequency value at the upper limit of the third quartiles of energy
	F1mean (Hz)	F2mean	Mean frequency value of the first formant
	F2mean (Hz)	F1mean, F3mean, F4mean	Mean frequency value of the second formant
F3mean (Hz)	F2mean, F4mean	Mean frequency value of the third formant	
F4mean (Hz)	F2mean, F3mean	Mean frequency value of the fourth formant	

1066
 1067 Correlations across individuals between a given parameter and others within its category
 1068 (physiological, behavioural or vocal) are indicated when significant (Spearman's rank
 1069 correlation; $P < 0.05$).

1070 **Table 3.** Effect of arousal and valence on physiological and behavioural
 1071 parameters.

Parameter	AROUSAL						VALENCE									
	0		1		χ^2_1 (N)	p	Negative		Neutral		Positive		χ^2_1 (N)	p		
	Mean	SD	Mean	SD			Mean	SD	Mean	SD	Mean	SD				
RMSSD	0.05	0.42	-0.07	0.40	7.05	0.008	>	0.01	0.42	0.03	0.41	-0.10	0.41	1.58	0.21	
RespRate	-0.10	0.28	0.17	0.26	76.67	<0.0001	<	0.05	0.32	-0.12	0.25	0.18	0.23	0.75	0.39	
HeadMov	-0.56	0.76	0.54	0.80	35.59	<0.0001	<	-0.23	0.70	-0.41	0.99	0.86	0.86	16.58	<0.0001	NC
Locomotion	-0.18	0.44	0.18	0.58	9.39	0.002	<	0.05	0.55	-0.17	0.42	0.08	0.61	0.00	0.96	
EarsForw	-0.03	0.09	0.03	0.08	11.36	0.0008	<	0.01	0.07	-0.10	0.07	0.06	0.06	2.14	0.14	
EarsHoriz	0.29	1.21	-0.57	0.63	15.43	<0.0001	>	-0.52	0.71	1.42	0.77	-0.74	0.04	0.21	0.65	
EarsBack	0.37	0.99	-0.15	1.20	4.56	0.033	>	0.63	0.77	-0.01	1.16	-0.91	1.03	31.50	<0.0001	>
EarsAsym	0.30	1.11	-0.24	1.17	4.56	0.033	>	0.32	1.11	0.34	1.10	-0.89	0.84	14.75	0.0001	NC
TailUp	-0.20	0.97	0.27	0.78	5.64	0.018	<	-0.25	0.92	0.16	0.90	0.50	0.68	10.78	0.001	<
Calls	-0.46	1.03	0.36	1.11	11.61	0.0007	<	0.23	1.13	-1.09	0.15	0.52	1.09	0.03	0.87	

1072

1073 Residuals of the models controlled for locomotion (physiology only), sex, age, individual
 1074 identity and breed (mean \pm SD; raw values are listed in Appendix Table A2), along with
 1075 statistical results (χ^2 values, sample size (*n*) and *p* values). The direction of the effect is
 1076 indicated (" $<$ " indicates an increase with arousal level or from negative to positive
 1077 valence, whereas " $>$ " indicates a decrease; NC indicates that the effect was not
 1078 consistent, i.e. increase followed by decrease or vice-versa). Significant results are
 1079 shown in bold.

1080 **Table 4.** Results of AIC comparisons for behavioural parameters significantly affected
 1081 both by arousal and valence.
 1082

Parameter	Arousal/ Valence	AIC _c	ΔAIC _c	w _i
HeadMov	A	215.47	0.00	1.00
	V	234.49	19.01	0.00
EarsBack	A	115.60	26.94	0.00
	V	88.66	0.00	1.00
EarsAsym	A	125.39	10.19	0.01
	V	115.20	0.00	0.99
TailUp	A	109.17	5.14	0.07
	V	104.03	0.00	0.93

1083
 1084 The best fit (arousal or valence based on lowest AIC_c) for a given response variable (set
 1085 of models) is indicated in bold. The fit of the models is assessed by Akaike's information
 1086 criterion corrected for small sample sizes (AIC_c). ΔAIC_c gives the difference in AIC_c
 1087 between each model and the best model. The Akaike's weights (w_i) assess the relative
 1088 support that a given model has from the data, compared to other candidate models in
 1089 the set.
 1090

1091 **Table 5.** Effect of arousal and valence on vocal parameters.

1092

Parameter	AROUSAL						VALENCE							
	0		1		χ^2_1 (N)	p	Negative		Positive		χ^2_1 (N)	P		
	Mean	SD	Mean	SD			Mean	SD	Mean	SD				
Duration	-0.01	0.13	0.00	0.11	0.35	0.55	0.00	0.12	-0.01	0.11	0.63	0.43		
F0mean	-0.02	0.10	0.01	0.10	4.67	0.031	<	0.00	0.11	0.01	0.10	0.23	0.63	
F0end	-0.05	0.19	0.03	0.15	13.36	0.0003	<	-0.01	0.17	0.04	0.15	3.43	0.06	
F0range	0.04	0.38	-0.02	-0.47	1.20	0.27		0.05	0.43	-0.16	0.43	6.88	0.009	>
FMextent	0.09	0.44	-0.04	0.59	3.20	0.07		0.05	0.52	-0.17	0.58	5.26	0.022	>
AMextent	0.01	0.43	0.00	0.31	0.03	0.85		-0.02	0.36	0.07	0.31	2.06	0.15	
Q25%	-20.02	88.36	10.01	73.00	7.10	0.008	<	-2.39	77.25	8.36	87.38	0.57	0.45	
Q50%	-0.10	0.37	0.05	0.28	12.50	0.0004	<	-0.01	0.33	0.05	0.29	1.25	0.26	
Q75%	-0.08	0.50	0.04	0.32	5.84	0.016	<	-0.01	0.40	0.04	0.35	0.68	0.41	
F1mean	40.15	106.75	-17.27	81.65	16.56	<0.0001	>	-4.37	94.77	15.69	87.77	1.07	0.30	
F2mean	11.58	152.13	-3.05	101.45	0.46	0.50		0.72	122.46	-2.35	77.90	0.02	0.90	
F3mean	-6.08	102.16	1.80	118.64	0.14	0.71		3.46	120.46	-11.11	94.95	0.36	0.55	
F4mean	-36.93	90.44	11.04	146.92	3.68	0.055	<	5.44	136.34	-19.16	141.12	0.68	0.41	

1093

1094 Residuals of the models controlled for sex, age, breed and call type (mean \pm SD; raw
 1095 values are listed in Appendix Table A2), along with statistical results (χ^2 values, sample
 1096 size (N) and p values). The direction is indicated for the significant and marginally
 1097 significant ($0.06 \geq p \geq 0.05$) effects (" $<$ " indicates an increase with arousal level or from
 1098 negative to positive valence, whereas " $>$ " indicates a decrease). Significant and
 1099 marginally significant results are shown in bold.

1100

1101 **Table A1.** Control factors and differences between Frustration and Isolation situations.

	Parameter	Sex		Age		Call type		Frustration vs Isolation	
		χ^2_1	<i>p</i>	χ^2_1	<i>p</i>	χ^2_1	<i>p</i>	χ^2_1	<i>p</i>
Physiology	RMSSD	0.17	0.68	0.12	0.73	-	-	3.60	0.058
	RespRate	8.15	0.004	3.79	0.052	-	-	27.44	<0.0001
Behaviour	HeadMov	0.01	0.94	3.22	0.07	-	-	26.53	<0.0001
	Locomotion	4.41	0.036	0.94	0.33	-	-	8.07	0.005
	EarsForward	3.37	0.07	0.00	1.00	-	-	0.70	0.40
	EarsHorizontal	0.20	0.65	0.02	0.89	-	-	1.15	0.28
	EarsBackward	0.17	0.68	0.77	0.38	-	-	4.64	0.031
	EarsAsymmetric	0.00	0.98	0.26	0.61	-	-	0.12	0.73
	TailUp	0.03	0.87	2.06	0.15	-	-	4.61	0.032
	Calls	0.38	0.54	2.45	0.12	-	-	0.03	0.86
Vocalisations	Duration	3.60	0.06	1.96	0.16	1.11	0.57	0.41	0.52
	F0mean	1.79	0.18	0.03	0.85	8.17	0.017	5.74	0.017
	F0end	3.10	0.08	0.39	0.53	2.09	0.35	8.44	0.004
	F0range	0.06	0.80	0.78	0.38	9.74	0.008	0.22	0.64
	FMextent	0.10	0.76	0.61	0.44	7.84	0.020	0.53	0.47
	AMextent	0.13	0.72	0.34	0.56	0.40	0.82	0.33	0.56
	Q25%	9.33	0.002	3.53	0.06	37.87	<0.0001	6.37	0.012
	Q50%	3.47	0.06	0.02	0.89	18.98	<0.0001	8.64	0.003
	Q75%	1.43	0.23	0.03	0.87	3.64	0.16	4.55	0.033
	F1mean	0.16	0.69	0.27	0.60	18.55	<0.0001	21.71	<0.0001
	F2mean	0.08	0.77	0.08	0.78	2.13	0.35	0.52	0.47
	F3mean	0.37	0.54	0.20	0.65	4.21	0.12	0.08	0.78
F4mean	0.34	0.56	0.12	0.73	2.80	0.25	4.02	0.045	

1102

1103 Effects of the control factors (age, sex and call type), as well as difference between the
1104 Frustration and Isolation situations (both assumed of negative valence), for physiological,
1105 behavioural and vocal parameters (linear mixed-effects models and generalized linear
1106 mixed models, compared with likelihood-ratio tests). Bold font indicates significant ($p <$
1107 0.05) and marginally significant ($0.06 < p \leq 0.05$) effects. The direction of the significant
1108 changes was assessed from residuals of the models. Sex (female, "F"; or male, "M")
1109 affected RespRate (F > M), Locomotion (F < M) and Q25 (F > M). Age tended to affect
1110 RespRate (marginally significant decrease with age). The type of call (closed mouth "CM",
1111 open mouth "OP" or mixed call "Mi") affected F0-related parameters (F0mean: Mi > OP >
1112 CM), the variation in F0 (F0range and FMextent: Mi > OP > CM), the energy quartiles

1113 (Q25% and Q50%: Mi > OP > CM) and F1mean (OM > Mi > CM). The difference between
1114 the Frustration ("Fr") and the Isolation situations ("I") was marginally significant for
1115 RMSSD (Fr < I), and significant for RespRate (Fr > I), HeadMov (Fr > I), Locomotion (Fr
1116 > I), EarsBackward (Fr < I) and TailUp (Fr > I). Concerning vocal parameter, this
1117 difference was significant for F0-related parameters (F0mean: Fr > I), the energy
1118 quartiles (Q25-Q75%: Fr > I), F1mean (Fr < I) and F4mean (Fr > I). In addition, the
1119 time spent moving (Locomotion) was included as a control factor for the physiological
1120 indicators. It had a significant effect on RespRate (LMM: $X^2 = 6.03$, $df = 1$, $p = 0.014$),
1121 but not on RMSSD (LMM: $X^2 = 0.09$, $df = 1$, $p = 0.77$).

1122

1123 **Table A2.** Raw values of the physiological, behavioural and vocal parameters measured
 1124 for each arousal level and valence of the situations (Mean \pm SD).

1125

1126		AROUSAL				VALENCE						
		0		1		Negative		Neutral		Positive		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Physiology	HR (beats/min)	108.75	15.38	123.00	23.52	115.74	21.94	107.79	12.25	126.37	24.71	
	RMSSD (ms)	31.66	19.65	26.83	15.21	29.90	18.53	31.21	19.49	26.15	13.34	
	RespRate (breaths/s)	0.32	0.10	0.42	0.11	0.38	0.13	0.31	0.08	0.42	0.09	
Behaviour	HeadMov (min ⁻¹)	7.16	10.61	22.02	26.08	9.73	13.48	9.68	14.95	29.80	31.17	
	Locomotion (%)	3.78	3.57	7.69	8.60	5.87	5.49	3.49	2.38	7.71	10.81	
	EarsForward (%)	61.57	29.80	82.02	27.04	74.80	25.97	41.93	26.79	92.54	19.14	
	EarsHorizontal (%)	16.25	25.08	0.21	1.11	0.25	1.14	35.99	26.32	0.00	0.00	
	EarsBackward (%)	12.63	14.18	15.37	21.04	19.72	17.74	9.13	14.88	6.39	17.62	
	EarsAsymmetric (%)	8.61	15.10	2.58	5.57	2.84	3.10	16.16	20.07	1.86	7.26	
	TailUp (%)	33.56	42.01	57.07	46.23	35.15	45.67	46.34	42.25	64.54	43.58	
	Calls (min ⁻¹)	0.45	1.59	3.36	4.40	1.55	2.74	0.00	0.00	4.67	5.16	
	Vocalisations	Dur (s)	0.84	0.21	0.78	0.16	0.81	0.19	-	-	0.78	0.15
		F0Mean (Hz)	253.40	105.98	228.30	56.56	240.42	81.58	-	-	223.51	58.76
F0End (Hz)		189.30	62.83	190.49	51.26	189.88	56.23	-	-	190.83	52.14	
F0Range (Hz)		115.75	84.85	91.29	52.63	106.75	69.21	-	-	73.85	44.95	
FMExtent (Hz)		71.16	42.52	75.37	105.98	79.99	99.20	-	-	52.87	36.92	
AMExtent (dB)		11.76	7.46	11.96	5.52	11.68	6.49	-	-	12.63	5.14	
Q25% (Hz)		241.83	135.94	255.42	109.55	251.98	120.39	-	-	247.09	114.39	
Q50% (Hz)		437.61	263.19	453.05	209.33	446.91	226.02	-	-	451.38	238.04	
Q75% (Hz)		1020.65	814.46	997.37	659.22	998.30	689.43	-	-	1029.04	797.02	
F1Mean (Hz)		694.97	163.87	655.17	113.25	659.00	132.24	-	-	696.33	125.15	
F2Mean (Hz)		1651.64	369.82	1596.72	289.56	1621.11	307.98	-	-	1566.03	305.97	
F3Mean (Hz)		2544.34	419.27	2549.80	368.42	2573.22	370.67	-	-	2469.27	400.37	
F4Mean (Hz)	3283.72	471.22	3423.91	521.30	3420.27	497.53	-	-	3290.92	557.30		

1127 **Audio A1. Negative and positive calls.** Audio files corresponding to the calls
1128 presented in Fig. 2; one call produced during the negative situation followed by a call
1129 produced during the positive situation by the same goat.