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Carole Fureix, Cleo Beaulieu, Soizic Argaud, Céline Rochais, Margaret Quinton, et al.. Investigating anhedonia in a non-conventional species: Do some riding horses *Equus caballus* display symptoms of depression?. *Applied Animal Behaviour Science*, 2015, 162, pp.26 - 36. 10.1016/j.applanim.2014.11.007 . hal-01101485

HAL Id: hal-01101485

<https://univ-rennes.hal.science/hal-01101485v1>

Submitted on 9 Jan 2015

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1 **Investigating anhedonia in a non-conventional species: do some riding**
2 **horses *Equus caballus* display symptoms of depression?**

3

4 Carole Fureix^{a1}, Cleo Beaulieu^a, Soizic Argaud^b, Céline Rochais^c, Margaret Quinton^a, Séverine
5 Henry^c, Martine Hausberger^{b,c}, Georgia Mason^{a*}

6

7 ^a Department of Animal and Poultry Science, University of Guelph, 50 Stone Road
8 East □ Guelph, Ontario, N1G 2W1, Canada

9 ^b CNRS UMR 6552 Ethologie Animale et Humaine, Université Rennes 1 Bâtiment 25, Campus
10 de Beaulieu, 263 Avenue du General Leclerc, CS 74205, 35042 Rennes Cedex, France

11 ^c Université Rennes 1 UMR CNRS 6552 Ethologie Animale et Humaine, Station Biologique
12 de Paimpont, 35380, Paimpont, France

13

14 * corresponding author: g.mason@uoguelph.ca Tel.: +44-0117-928-9485

¹ Present address: Centre for Behavioural Biology, Department of Clinical Veterinary Science, University of Bristol, Langford House, Langford BS40 5DU, UK

15 ABSTRACT

16 Investigating depression-like conditions in animals is methodologically challenging, but
17 potentially important for welfare. Some riding horses display ‘withdrawn’ states of inactivity
18 and low responsiveness that resemble the reduced engagement with the environment shown by
19 certain depressed patients. To assess whether these animals are experiencing a depression-like
20 state, we investigated anhedonia -- the loss of pleasure, a key symptom of human depression -
21 - in 20 withdrawn and non-withdrawn horses from the same stable. The time horses spent being
22 withdrawn appeared unrelated to age or sex, but correlated with time devoted to stereotypic
23 behaviour, a possible marker of lifetime stress. Comparison with data collected 5 years earlier
24 also revealed that horses scored as withdrawn then remained significantly likely to display the
25 behaviour. We measured sucrose intake, a classic measure of anhedonia never previously
26 applied to horses. Flavoured sugar blocks, novel to these subjects, were mounted in each stall
27 and weighed 3h, 8h, 24h and 30h after provision. We predicted that if affected by depression-
28 like states, the most withdrawn horses would consume the least sucrose. This prediction was
29 met ($F_{1,18} = 4.65$, two tailed $p = 0.04$). This pattern could, however, potentially reflect general
30 appetite levels and/or food neophobia. To control for these confounds, hay consumption was
31 measured over 5 days, as were subjects’ latencies to eat a meal scented with a novel odour.
32 Although low hay consumption and long latencies to eat scented food did predict low sucrose
33 consumption, statistically controlling for these confounds did not eliminate the relationship
34 between being withdrawn and consuming less sucrose (although reducing it to a strong trend):
35 $F_{1,15} = 4.28$, two-tailed $p = 0.056$. These data thus suggest long-lasting depression-like states in
36 certain riding horses, which correlate with stereotypic behaviour and are characterised by
37 anhedonia and bouts of ‘withdrawn’ unresponsiveness.

38

39 Key-words: horses; depression; anhedonia; depression-like conditions; reduced engagement
40 with environment; DSM-V

41 1. INTRODUCTION

42 In humans, clinical depression -- by which we mean “major depressive disorder” or the
43 experience of “depressive episodes”, to encompass DSM-V (Diagnostic Manual of Mental
44 Disorders- fifth edition, American Psychiatric Association APA, 2013) and ICD-10
45 (International Statistical Classification of Diseases and Related Health Problems, World Health
46 Organisation WHO, 1994) terminologies – is a common mental illness (*e.g.* annual prevalence
47 in the U.S.: 7% of the population, APA, 2013), especially in women. It is a complex,
48 phenotypically heterogeneous syndrome, clinically diagnosed by the co-occurrence of a variety
49 of affective, cognitive and behavioural symptoms (Table 1) that include low, hopeless moods,
50 “not caring”, social withdrawal, and fatigue (APA, 2013). These are present for many days or
51 weeks, and interfere with abilities to cope with everyday life. Related phenomena supporting
52 diagnosis include anxiety, tearfulness, obsessive rumination, brooding, and complaints of aches
53 and pains (APA, 2013), with one study further highlighting “nonspecific gaze, withdrawal, [*no*
54 *mouth movements, and*], no eye region movement” as behavioural markers of the condition
55 (Schelde, 1998). Additional attributes common in clinically depressed humans – although not
56 reliable, specific or sensitive enough for use in diagnosis (APA, 2013) – include alterations of
57 the hypothalamic-pituitary-adrenal (HPA) axis (both elevations *e.g.* Miller et al., 2007, and sub-
58 normal levels *e.g.* Strickland et al., 2002).

59 In terms of aetiology, a common trigger is chronic stress, for instance that arising from
60 aversive life events or chronic pain or illness (*e.g.* Banks and Kerns, 1996; Blackburn-Munro
61 and Blackburn-Munro, 2001; Tafet and Bernardini, 2003; Munce et al., 2006; Siegrist, 2008;
62 Hammen et al., 2009; APA, 2013). Two types of cognitive change can often be observed before
63 the illness fully develops, and these may act as mediators in some subjects, being hypothesised
64 to help cause the onset and/or maintenance of the disease (Beck, 1967; Gotlib and
65 Krasnoperova, 1998). One is ‘learned helplessness’, which is proposed to occur “when highly

66 desired outcomes are believed improbable or highly aversive outcomes are believed probable,
67 and the individual comes to expect that no response in his repertoire will change their
68 likelihood” (Abramson et al., 1978). The second involves negative biases in attention, memory
69 and/or judgment (Beck, 1967; MacLeod and Byrne, 1996; Gotlib and Krasnoperova, 1998).
70 Thus depressed people are prone for example, to judge ambiguous stimuli as being unlikely to
71 be positive (‘cognitive pessimism’), and to recall unpleasant memories more readily than
72 pleasant ones.

73 The symptoms of depression may not be unique to humans. A Web of Science literature
74 search using the terms “rats” OR “mice” OR “monkeys” AND “depression” yielded over
75 100,000 articles: laboratory rodents in particular are widely used to model clinical depression,
76 primarily to screen drugs for human use (reviewed in *e.g.* Matthews et al., 2005; Deussing,
77 2006). Although “it is exceedingly difficult to envision an animal model that perfectly
78 recapitulates the symptoms of depression in human patients” (Deussing, 2006), much of this
79 animal work does appear to validly model at least some symptoms of this disease. For an animal
80 condition to be deemed homologous to a human illness, it must display several forms of validity
81 (Belzung and Lemoine, 2011; Camus et al., 2013a): symptoms should seem analogous to those
82 of affected humans (sometimes called “face validity”); the animal’s condition should mimic
83 the human disease in terms of risk, protective and therapeutic factors (sometimes called
84 “predictive validity”); and underlying mechanisms should ideally be homologous (although for
85 depression, this would require a consensus about its mechanistic bases that does not currently
86 exist, see *e.g.* Moore, 2002; Strickland et al., 2002). By exposing laboratory animals to chronic
87 unpredictable stress – as well as to lesions, drugs and genetic manipulations that arguably have
88 less predictive validity (Deussing, 2006) – researchers have successfully induced responses
89 that have face validity with certain features of human depression: “helpless” reactions to

90 unavoidable stressors (*e.g.* Maier and Seligman, 1976), along with anhedonia (*e.g.* Willner et al.,
91 1992), a reduction in pleasure that we discuss in detail below.

92 Furthermore, outside of this type of research environment, circumstantial evidence has
93 led several authors to hypothesise that depression-like states occur in other animals. Pet dogs
94 and cats have been suggested to show “depressed behaviour” when deprived of their owners
95 (Fox, 1968, p. 357), as have apes housed long-term in barren environments in laboratories or
96 zoos (*e.g.* Engel, 2002 p174, Brune et al., 2006; Ferdowsian et al., 2011; Hennessy et al., 2014)
97 and maternally deprived monkeys (*e.g.* Harlow and Suomi, 1974; Suomi et al., 1975; Hennessy
98 et al., 2014). Horses, too, have been suggested to sometimes display depression-like symptoms
99 (Pritchard et al., 2005; Hall et al., 2008; Burn et al., 2010; Fureix et al., 2012; Popescu and
100 Diugan, 2013). States involving profound inactivity and low responsiveness to external stimuli
101 have thus been reported in working equids in the developing world (Swann, 2006; Burn et al.,
102 2010), and in riding horses in Europe and North America (Fureix et al., 2012). For example,
103 Hall and colleagues describe riding school horses “who seem to have ‘switched off’ (are
104 unresponsive, lack motivation, and are apathetic)”. Recently, data have been collected to
105 describe such horses’ characteristics more formally. Fureix et al. (2012) found that ‘withdrawn’
106 states are characterised by a stationary, atypical, flat-necked posture (**Figure 1**); wide open,
107 unblinking eyes with an apparently fixed gaze; and backwards-pointing ears. Even when not
108 engaged in these unusual behaviours, horses with these states differed from non-withdrawn
109 horses from the same stable in several ways: they show reduced responsiveness to tactile
110 stimulation; less reaction to a human’s sudden appearance at the stall door; less exploration
111 and more behavioural signs of arousal (fear) when exposed to a novel object; and lower
112 baseline levels of plasma cortisol. Furthermore, while the full aetiology of such states is still
113 unclear, being withdrawn was more prevalent in female riding horses than male (Fureix et al.,
114 2012); inactivity combined with low responsiveness to external stimuli is, in working equids,

115 associated with illness and advanced age (Burn et al., 2010); and, more speculatively, sustained
116 adversity has been hypothesised to play a role. Thus Hall et al. (2008) suggested that “apathetic
117 and lethargic” horses have generally been “stabled for the majority of their lives with no
118 opportunity for social interaction”, with such responses reflecting stress-induced “learned
119 helplessness” (see also Ödberg, 1987). Fureix et al. (2012) therefore hypothesised, by analogy
120 with human clinical depression, that withdrawn horses exhibit a depression-like state.

121 We chose to test this hypothesis empirically for horses, by assessing face validity more
122 formally *via* the measurement of anhedonia. Recognised as symptom of depression for over 40
123 years (Feighner et al., 1972), anhedonia has been a diagnostic criterion since DSM-III (APA,
124 1980), and is now seen as one of the condition’s most important symptoms (WHO, 1994; APA,
125 2013; Table 1). In animals, anhedonia has been successfully modelled in biomedical studies of
126 rodents, primarily *via* inducing and recording reductions in sucrose intake (*e.g.* Papp et al., 1991;
127 Willner et al., 1992; Forbes et al., 1996; Brennan et al., 2001; Deussing, 2006; Walker and
128 Mason, 2011). Evidence that this sucrose-ingestion is pleasure-driven includes that rodents will
129 eat sugar even when fully sated (*e.g.* Jarosz et al., 2006; Lowe and Butryn, 2007; Pratt et al.,
130 2012); that it involves the same opioid-mediated reward pathways as sexual behaviour and
131 some recreational drugs (*e.g.* Lowe and Butryn, 2007; Olsen, 2011; Pratt et al., 2012); and that
132 in rats the frequency of 50 KHz ultrasonic vocalisations (typically produced when anticipating
133 positive reinforcers; Knutson et al., 2002) correlates positively with preferences for sweet food
134 (Mateus-Pinheiro et al., 2014). That reduced sucrose intake by rodents is a symptom of
135 depression-like anhedonia has been further validated by its induction by chronic stressors (*e.g.*
136 Papp et al., 1991; Willner et al., 1992; Gronli et al., 2005); its alleviation by anti-depressant
137 drugs (*e.g.* Muscat et al., 1992; Nestler et al., 2002; Deussing, 2006; McArthur and Borsini,
138 2006; Malatynska et al., 2012); and its co-variation with other depression-like features
139 including learned helplessness (*e.g.* Strekalova et al., 2004) and negative cognitive biases

140 (Rygula et al., 2013). Like rodents, horses reportedly prefer sucrose solutions to water (Randall
141 et al., 1978), and often choose sweetened food over unsweetened (Hawkes et al., 1985). We
142 therefore chose to assess reduced sucrose intakes as a measure of anhedonia in horses.

143 This work thus aimed to use this measure to test the hypothesis that withdrawn horses
144 are experiencing a depression-like condition. Using withdrawn and non-withdrawn horses from
145 the same riding stable, we predicted that, if in depression-like states, withdrawn horses would
146 consume less sucrose than non-withdrawn stable-mates. To both refine this experiment and
147 better characterise the withdrawn state, we also assessed some additional variables. Most of
148 our subjects had been screened for withdrawn states five years previously (Fureix et al., 2012);
149 we therefore opportunistically investigated whether individuals displaying withdrawn postures
150 in 2007 would still be prone now. In the absence of direct information on the types of aversive
151 early life experience and stressful life events that predispose humans to depression (*e.g.* Gilmer
152 and McKinney, 2003; Tafet and Bernardini, 2003; Siegrist, 2008), we also assessed our
153 subjects' levels of stereotypic behaviour. In populations with diverse past experiences,
154 stereotypic behaviour, although definitely not a symptom of depression, is typically most
155 prevalent or severe in individuals that have experienced challenges during early development
156 and/or stressful lives since (Lutz et al., 2003; Mason and Latham, 2004; Jones et al., 2011;
157 Gottlieb et al., 2013). Assuming this holds for horses, we used stereotypic behaviour as a proxy
158 measure of individuals' life stress, predicting positive relationships between it and the display
159 of withdrawn states. Finally, we checked for three additional confounds that, while not
160 themselves markers of depression, could potentially mediate a positive relationship between
161 withdrawn behaviour and sucrose intake. The first was general appetite at the time of test:
162 checking that general feed consumption levels do not explain sucrose consumption is an
163 important control in rodent and primate biomedical studies of anhedonia (*e.g.* Willner et al.,
164 1992; Paul et al., 2000). The second was food neophobia. The forms of sucrose we used were

165 novel to the subjects (see Methods); withdrawn horses appear to react to novelty more strongly
166 than non-withdrawn horses (Fureix et al., 2012); and in laboratory mice, neophobia increases
167 the latency with which mice ingest solid sugar placed in their cages, independently of
168 anhedonia (Walker and Mason, 2011). We predicted that if in depression-like states, withdrawn
169 horses should still consume less sucrose than non-withdrawn horses even after statistically
170 accounting for these two potential confounds. The third potential confound was withdrawn
171 horses not noticing the offered sucrose because engaged in bouts of non-responsiveness. We
172 therefore assessed sucrose consumption in the first 3 hours after presentation, to check that
173 both withdrawn and non-withdrawn horses sampled it within that short time.

174

175

176 2. METHODS

177

178 2.1. Ethical note

179 This study was approved by the University of Guelph Animal Care Committee (Animal
180 Utilization Protocol number: 2023) and complied with the Canadian Council on Animal Care
181 guidelines, French laws related to animal experimentation and the European directive
182 86/609/CEE. Horse husbandry and care were under management of the riding school: the
183 horses used in this experiment were not research animals.

184

185 2.2. Subjects

186 Twenty horses –16 geldings and 4 mares, aged 7 to 20 years old (on average 14.5 ± 3.9)
187 – were observed between June and November 2012 (**Table 2**). Fifteen had been already studied
188 five years previously (Fureix et al., 2012) (**Table 2**). All came from a single riding school
189 located in western France, at which they had been housed for at least a year. The sample

190 included 17 French Saddlebreds, one French Trotter plus one Anglo-Arabian (two breeds
191 originally involved in the creation of the French Saddlebred breed) and one unregistered horse
192 (thus of unknown breed). Each was kept singly in 3 m x 3 m individual straw-bedded stall in a
193 barn, allowing visual contact with conspecifics. Each stall was cleaned every morning, and was
194 equipped with an automatic drinker. Animals were fed hay (6-7kg) once daily (13:00h), and
195 commercial pellets three times daily (07:00h, 12:00h, 19:00h). These pellets were composed
196 of wheat bran, (30%), barley (28%), alfalfa flour (10%), palm kernels (10%), soya beans
197 (10%), oats (6%), and trace amounts of treacle, corn, calcium carbonate, sodium chloride,
198 vitamins A, D, E and copper sulphate. The horses worked in riding lessons for 4 to 12 hours a
199 week, with two rest days per week during which they were released in groups into paddocks.
200 Riding lessons involved children and teenagers, and both indoor (instruction) and outdoor
201 activities, including a few competitions.

202

203 *2.3. Behavioural recording: withdrawn status and stereotypic behaviour*

204 The time each horse spent being withdrawn in its stall was determined by a single
205 trained observer, using instantaneous scan sampling (Martin and Bateson, 2007) every 2
206 minutes over 1h long periods, repeated daily over 15 days. Sampling sessions were conducted
207 during daylight, at 06:00h, 07:00h, 10:00h, 11:00h, 15:00h, 16:00h, 20:00h and 21:00h. The
208 silent observer (CB) walked regularly along the corridor and observations were made at a
209 distance of 3m. The average number of total scans obtained per subject was 907 ± 11.14 (horses
210 were sometimes away from their stall, *e.g.* for lessons, resulting in variation in observation
211 number). All behaviours were recorded (see *e.g.* Waring, 2003 for a detailed ethogram), but we
212 only report here only the states of interest for this study, *i.e.* the time spent being withdrawn,
213 along with stereotypic behaviour (crib-biting, tongue and lips movements, weaving, head
214 shaking and nodding; descriptions in **Table 3**; see also *e.g.* Hausberger et al., 2009; Fureix et

215 al., 2011a). The fully withdrawn state was defined in accordance with Fureix et al. (2012), as
216 follows: the horse was standing motionless, with eyes open but unblinking, without ear or head
217 movements, and displaying a stretched neck (*i.e.* obtuse jaw-neck angle) and a similar height
218 between neck and back (*i.e.* a nape–withers–back angle of 180°) (see also **Fig 1**). A horse
219 displaying all of the above-mentioned characteristics, but blinking the eyes or moving the ears
220 once, was scored as “moderately withdrawn”. We chose to do so due to a lack of control over
221 aspects of the environment, such as very windy weather or unpredictable loud noises in the
222 stable, that could potentially very briefly disrupt withdrawn states by inducing a very brief ear
223 movement or eye blinking (therefore violating, although very briefly, the withdrawn definition
224 criteria). Spending time fully withdrawn was extremely strongly related to spending time
225 moderately withdrawn (see Results), supporting *a posteriori* our assumption that the two
226 measures reflected the same state, and we therefore pooled the two measures in the ethogram
227 for further analyses. Note that *sensu stricto* instantaneous scan sampling was unsuitable for
228 identifying withdrawn states, which were characterised by a lack of movement, and for
229 stereotypic behaviours characterised by repetition. In such instances, the observer switched to
230 focal observation, the activity of the horse of interest being continuously observed for 15 s (see
231 *e.g.* Mason, 1993 and Tilly et al., 2010 for a similar mixture of focal and instantaneous scan
232 sampling).

233

234 *2.4. Sucrose intake measurement*

235 Over the six days prior to the study, all horses were offered 10g of raw sugar (lumps)
236 once a day, presented by the observer directly under the nostrils, palm up. All horses consumed
237 sugar lumps, revealing no spontaneous aversion towards sucrose in any of the subjects (see *e.g.*
238 Hawkes et al., 1985 for evidence that sucrose consumption is common but not universal in
239 horses). On the first day of the experiment, flavoured sucrose blocks (600g, Likit®, UK), all

240 novel to these horses, were presented under the nostrils of each subject until one lick occurred,
241 and then immediately mounted on the wall of each stall like a salt block. To measure rates of
242 sucrose intake, these sugar blocks were weighed 3h, 8h, 24h and 30h after provision (initial
243 time of block mounting: between 09:00 and 11:00). The 30h long testing period covered both
244 pre- and post-meals intervals, allowing us to control for alliesthesia (modulation of the
245 rewarding value of food according to whether in a deprived or satiated state, Booth et al., 1972;
246 Thompson et al., 1976). To control for possible individual variation in flavour preference
247 (known in horses, Goodwin et al., 2005), the test was replicated three times in succession, using
248 three different Likit® flavours (cherry, banana and apple). Flavour order was pseudo-randomly
249 assigned to control for effects of presentation order.

250

251 *2.5. Hay consumption and the “novel meal” experiment*

252 All subjects’ total hay consumption over a 5 day period was measured to estimate
253 overall food consumption level. Hay nets containing the usual rations were weighed daily pre-
254 feeding (at 13:00h) and after 3h of exposure. Any fallen hay was collected from the bedding
255 and placed back in the hay net prior to each weighing. To assess neophobia towards novel
256 foods, horses’ latencies to eat a meal of their usual pellets that had been scented with a mixture
257 of unfamiliar aromatics (10g of aromatic herbs “Herbes de Provence” and 10 ml of a solution
258 “Arôme Fleur d’Oranger”, Vahiné®) were then measured. Long latencies to interact with a
259 novel object are typically interpreted as reflecting higher levels of anxiety or neophobia (*e.g.*
260 Wolff et al., 1997; Ennaceur et al., 2005). The aromatics and their concentrations were chosen
261 after pilot observations performed on five other horses from the same stable (not study subjects)
262 ensured that the novel flavour in food was perceptible (*e.g.* inducing sniffing) to horses. Meal
263 delivery was performed by the horses’ usual caretaker, following the normal daily routine but
264 with an experimenter by her side. The experimenter for this part was SA, not CB, and blind to

265 all horses' results from any other test/observation. Horses were tested one at a time,
266 counterbalanced according to their withdrawn status (*i.e.* testing equal proportions of
267 withdrawn and non-withdrawn horses) across time periods within a day (meal at 07:00h,
268 12:00h am or 19:00h) and over testing days. The experimenter began to record time
269 immediately after the caretaker poured the pellets into the feeding trough, stopping
270 immediately after the horse took two consecutive mouthfuls without any intervening behaviour
271 other than chewing and swallowing. This caretaker was also blind to the aims of the study,
272 being told that only horses' behaviours were being recorded for further analyses, and asked to
273 behave in her usual way. Each tested horse's latency to eat a normal meal of usual pellets was
274 also recorded (once per horse).

275

276 2.6. Statistical analyses

277 Data collected to test our hypothesis' main prediction were the percentage of time each
278 horse spent being withdrawn and the cumulative proportion of sugar consumed from the Likit®
279 600g blocks after 3h, 8h, 24h and 30h of exposure, averaging the "cherry", "banana" and
280 "apple" replicates. Data which were not hypothesized to be markers of depression-like
281 conditions *per se* but instead were informative about chronic stress and/or allowed us to rule
282 out potential confounds were: hay consumption (kg) over 5 days; latencies (s) to eat a meal of
283 normal pellets scented with novel aromatics; and the percentage of time each horse spent in
284 stereotypic behaviour. Descriptive statistics are means (X) followed by standard deviations,
285 and range (minimum/maximum).

286 For the fifteen subjects categorised five years previously as withdrawn or not (Fureix
287 et al., 2012), we assessed whether they still fell into the same categories (displaying at least
288 one withdrawn bout or never observed being withdrawn) using a Fisher's Exact test (Graphpad:
289 <http://graphpad.com/quickcalcs/contingency1.cfm>).

290 Simple general linear models (GLM) were conducted in JMP® 10 (SAS Institute Inc.,
291 Cary, NC, USA) (using an alpha of 0.05, and two-tailed tests) to investigate relationships
292 between time spent in withdrawn states (controlling for age and sex) and sugar consumption
293 and stereotypic behaviour; and between time spent in withdrawn behaviour and age and sex.
294 Normality and homogeneity of variances were assessed by inspection of residuals (Grafen and
295 Hails, 2002), and Shapiro-Wilk W tests. For these, and the subsequent models using SAS,
296 proportional data were always arcsinsquareroot transformed (Doncaster and Davey, 2007, p16)
297 to fit the parametric assumptions of GLMs.

298 The relationship between the time each horse spent being withdrawn and sucrose intake
299 was investigated using the following repeated measures mixed GLM (Model 1), accounting for
300 horse within sex as a random effect: *sugar eaten = horse(sex) + weighing time (3, 8, 24, 30h*
301 *after provision) + time spent withdrawn + age + sex + time each horse spent in its stall during*
302 *testing*. The time each horse spent in its stall was included because horses removed from their
303 stall for lessons obviously could not ingest sugar during those periods. The Brown and Forsythe
304 (modified Levene) test was used to compare the variances between treatment groups for all
305 variables prior to analysis, and showed no evidence of heterogeneity for any variable. Models
306 were run using the mixed procedure in SAS (version 9.3), and repeated measures were handled
307 according to the method given by Wang and Goonewardene (2004). The Akaike (1974)
308 criterion was used to determine the best fitting covariance structure among the repeated
309 measurements over time on the same horse, and the error degrees of freedom for hypothesis
310 testing was calculated using a Satterthwaite approximation. Residual plots were examined after
311 the analysis, and showed no evidence of any associations with means (or non-homogeneity of
312 variances).

313 Because this model revealed a significant relationship between withdrawn behaviour
314 and sucrose intake (see Results), both hay consumption and latency to eat the novel-scented

315 food were then added as to the model as additional controls (Model 2). Because stereotypic
316 behaviour co-varied with time spent withdrawn (see Results), one final repeated measures
317 model (with additional controls as Model 2) was conducted to assess whether stereotypic
318 behaviour *per se* predicted sucrose consumption. If it did not, any relationship between
319 anhedonia and withdrawn states could not be causally related to stereotypic behaviour (while
320 if it did, a model including both stereotypic behaviour and time spent being withdrawn as
321 predictors would be necessary in order to identify the best predictor of sucrose consumption).

322

323

324 3. RESULTS

325

326 Our subjects spent far more time being moderately withdrawn than fully withdrawn
327 (fully: $0.050 \pm 0.11\%$ time, range 0-0.40; moderately: $0.67 \pm 0.95\%$ time, range 0-0.29).
328 However, spending time fully withdrawn was extremely strongly related to spending time
329 moderately withdrawn ($F_{1,16} = 31.66$, $p < 0.0001$), and we therefore pooled the two measures.
330 The overall total proportion of time spent withdrawn (full + moderate) varied between 0 and
331 3.1% of observation time ($X = 0.72 \pm 1.05$), with nine animals never displaying the behaviour.
332 Horses that had been classified as withdrawn in 2007 were significantly more likely to display
333 withdrawn behaviour in this study (Fisher's exact test, two-tailed: $p = 0.041$). The time spent
334 withdrawn was unaffected by horse age ($F_{1,17} = 0.011$, $p = 0.917$) or sex ($F_{1,17} = 1.346$, $p =$
335 0.262), but was strongly related to the time ($X = 1.68 \pm 4.27\%$ time, 0-18%) devoted to
336 stereotypic behaviour ($F_{1,16} = 9.404$, $p = 0.007$).

337 In the Likit® ingestion tests, all but one horse ate the sugar (inferred from the weight
338 of the block declining over time in a stall). Eighty per cent of subjects began to eat within the
339 first 3h of exposure; the other 20% comprised two withdrawn and two non-withdrawn horses.

340 Withdrawn horses thus did not seem less likely to perceive the Likit® block. Furthermore, as
341 predicted, horses spending the most time withdrawn showed less sucrose intake (Model 1: $F_{1,18}$
342 = 4.65, $p = 0.04$) (**Fig 2**).

343 The potential confounds of general appetite and food neophobia were then investigated.
344 Total hay intake over five days varied from 19 to 34kg per horse ($X = 25.61 \pm 3.56$). *A*
345 *posteriori* comparisons confirmed longer latencies to eat the pellet meal scented with novel
346 aromatics ($X = 10.5s \pm 9.6$, 1-42s) than the usual meal ($X = 1.8s \pm 0.41$, 1-2s) (matched-pair *t*
347 test, $t_{19} = 4.08$, $p < 0.001$), showing that horses did perceive the novel-scented meal as
348 unfamiliar. Since latencies to eat the usual meal were all very short, never exceeding 2s, and
349 showed negligible variation between horses, all further analyses use the raw latencies to eat the
350 novel meal. When included in the repeated measures analysis (Model 2), low hay consumption
351 was found to predict low sucrose intake ($F_{1,14} = 4.52$, $p = 0.051$), as did long latencies to eat
352 the novel-scented food ($F_{1,14} = 8.34$, $p = 0.012$). However, statistically controlling for these two
353 confounds did not eliminate the previous negative relationship between spending time
354 withdrawn and eating sucrose (**Fig 3**), although reducing it to a strong trend ($F_{1,15} = 4.28$, $p =$
355 0.056).

356 Stereotypic behaviour, in contrast, did not predict sucrose consumption ($F_{1,15} = 0.780$,
357 $p = 0.3916$), despite being a strong correlate of time spent withdrawn.

358

359

360 4. DISCUSSION

361

362 As reviewed in the Introduction, our hypothesis that certain horses display depression-like
363 states (*e.g.* Swann, 2006; Hall et al., 2008; Burn et al., 2010; Fureix et al., 2012) was based on
364 apparent similarities between their reduced responsiveness and the reduced interactiveness of

365 some depressed human patients; evidence of associated anxiety, which would support a
366 diagnosis of depression in humans; hypocortisolaemia, as found in a sub-set of depressed
367 humans; and some preliminary signs of predictive validity (greater occurrence in females and
368 individuals suffering ill-health). To this, we now add evidence of a symptom that has strong
369 face validity with a DSM-V diagnostic criterion, and that has been successfully used in
370 biomedical research on depression-like states in rodents. Assuming that sucrose consumption
371 in horses is driven by pleasure, as in humans and rodents (Jarosz et al., 2006; Lowe and Butryn,
372 2007; Olsen, 2011; Pratt et al., 2012), then horses that display states of withdrawn reduced
373 reactivity are more anhedonic than those that do not. Such loss of pleasure is a key symptom
374 of clinical depression, and suggests that withdrawn horses are indeed in a depression-like state.

375 Our data also revealed that tendencies to be withdrawn, like clinical depression, are
376 possibly long-lasting: many of our subjects classified as withdrawn five years earlier still
377 displayed withdrawn behaviour now (although we cannot say whether their withdrawn
378 behaviours occurred persistently over that time). Furthermore, these withdrawn states covaried
379 with stereotypic behaviour. If individual differences in equine stereotypic behaviour reflect the
380 number and severity of aversive past experiences (as in primates: see Introduction), this
381 cautiously suggests that withdrawn states do likewise: an important hypothesis to test more
382 fully in the future since, if supported, it would add to evidence of aetiological similarities
383 between withdrawn states in horses and depressed states in humans.

384 All these findings still do not demonstrate with certainty that horses in human care can
385 become clinically depressed: the quality and quantity of current evidence are not yet sufficient
386 to conclude this. However, these data are sufficiently consistent with this hypothesis to make
387 additional research very worthwhile, especially into the breadth and depth of anhedonia in
388 affected horses, the existence of other key symptoms listed by the DSM, and the factors that
389 trigger or reverse the appearance of withdrawn states. Given the range of species and number

390 of individuals it has been suggested may be affected – not just horses but also captive primates
391 and perhaps even pet dogs and cats – the welfare implications of animal depression provide a
392 clear rationale for conducting such research. To end we therefore discuss how future research
393 into animal depression might progress, starting with the assessment of pleasure and its loss.

394 We chose to assess anhedonia *via* sucrose consumption because of its successful, valid
395 application in laboratory rodents, and ease of use for horses (important in a busy commercial
396 riding stable). Despite these advantages, however, our approach had two drawbacks. The first
397 is that a reduced interest in sugar is not, on its own, a symptom of clinical depression: anhedonia
398 refers to a “markedly diminished ... pleasure in **all, or almost all**, activities” (APA, 2013; our
399 emphasis). To demonstrate generalised anhedonia, the performance of a *range* of activities
400 motivated by positive affect must therefore be observed: perhaps affiliative behaviours with
401 preferred social partners (Ferdowsian et al., 2011), sexual behaviour (Gronli et al., 2005; Olsen,
402 2011), or interest in cues from potential mates (Finger et al., 2011). A second drawback was
403 that our assumption that horses’ sucrose ingestion is motivated by pleasure is untested. Both
404 problems highlight a broader issue: how little is still known about positive affective states and
405 hedonic motivations in almost all animal species (Boissy et al., 2007; Yeates and Main, 2008;
406 Mellor, 2012). Potential future tools for identifying such behaviours include their response to
407 naloxone – pleasure-motivated activities are more suppressed by opioid-antagonists than are
408 activities motivated by need (Van Ree et al., 2000; Boissy et al., 2007; Lowe and Butryn, 2007)
409 – and their response to deprivation: hedonically-motivated behaviours seem more driven by
410 opportunism and external stimuli (*i.e.* eliciting cues such as odours) than by states of
411 deprivation, while need-based behaviours are more sensitive to degrees of satiety or
412 physiological deficit (Fraser and Duncan, 1998).

413 Along with assessing anhedonia more fully in potentially depressed animals, a further
414 research need is to operationalise additional symptoms of depression for application to non-

415 humans. Diagnoses of clinical depression in humans require the presence of at least five of the
416 symptoms listed in Table 1, including anhedonia and/or low mood, plus evidence that they are
417 debilitating. We agree with Ferdowsian et al. (2011) in their recent discussion of depression-
418 like states in captive chimpanzees, that the DSM represents a clear, objective way to diagnose
419 depression in animals, but that two of its criteria (those pertaining to guilt and suicidality: items
420 7 and 9 in Table 1) are not operationalisable in non-humans. We also argue that a third,
421 psychomotor retardation or agitation (item 5), should not be applied to animals, because
422 identifying the former relies on speech, while the latter could be confused with stereotypic
423 behaviour: definitively not a symptom of human depression. This leaves six symptoms
424 (including anhedonia), of which five must be observed to trigger a diagnosis if DSM procedures
425 are followed: low mood (1); changes in weight and/or appetite (3) insomnia/hypersomnia (4);
426 fatigue or loss of energy (6); and reduced attentional, cognitive or decision-making abilities
427 (8). If conducted carefully and critically, all could be objectively quantified in animals. To
428 assess low mood, a particularly important feature of depression whose affective nature could
429 make assessment challenging, we suggest assessing ‘irritability’ (*e.g.* unprovoked aggressive
430 acts to conspecifics), as well as negative cognitive biases, such as pessimistic interpretations
431 of ambiguous scenarios that closely relate to low moods in clinically depressed people (Beck,
432 1967; MacLeod and Byrne, 1996; Gotlib and Krasnoperova, 1998), and that can be assessed in
433 animals (Harding et al., 2004; Mendl et al., 2009). As for evidence of dysfunction or
434 impairment, valid measures in animals might include reduced attractiveness to potential mates
435 (cf. Diez-Leon et al., 2013) and/or inadequate maternal care (Meagher et al., 2012).

436 The third crucial avenue of research is to investigate predictive validity: the aetiology
437 and response to therapeutic agents of depression-like conditions in horses and other animals.
438 Since aversive early life experiences and stressful life events act as risk factors in humans (*e.g.*
439 Gilmer and McKinney, 2003; Tafet and Bernardini, 2003; Siegrist, 2008), they might therefore

440 do so in animals (see *e.g.* Camus et al., 2013b). In working horses, variables to investigate should
441 include weaning age/practices (*e.g.* Visser et al., 2008); time spent in isolation (*e.g.* McGreevy et
442 al., 1995; Cooper et al., 2000); painful conditions like vertebral problems (*e.g.* McGreevy and
443 McLean, 2005; Hausberger et al., 2009; Lesimple et al., 2010); and exposure to stressful
444 working conditions (*e.g.* Hausberger et al., 2009; Lesimple et al., 2010; Hausberger et al., 2011).
445 How depression-like states in animals respond to social support, anti-depressant drugs and
446 reduced stress should also be investigated. Such work would not only fully test the predictive
447 validity of depression-like states in animals (cf. Belzung and Lemoine, 2011), but also have
448 significant welfare implications by revealing how we can we best prevent and treat such states.

449

450

451 5. CONCLUSION

452

453 Animals in human care sometimes display states of unresponsive inactivity after known
454 or suspected chronic stress, pain or social isolation, leading to suggestions that they are
455 ‘depressed’. Testing such hypotheses is important for animal welfare. We propose that
456 depression-like states in animals could be objectively identified using some of the diagnostic
457 criteria relied on by human clinicians. Our findings that riding horses that display bouts of
458 withdrawn inactivity (along with other depression-like changes) also consume less sucrose
459 under test, suggest that these animals are anhedonic. We urge for more research into the
460 assessment of pleasure and its loss in non-human animals.

461

462

463 6. ACKNOWLEDGMENTS

464 The authors are grateful to the director of the riding school and the staff for allowing us
465 to work with their horses and their help and understanding. This work was supported by a

466 Fyssen Foundation postdoctoral fellowship (to CF), a grant from the French Canada Research
467 Fund (held by GJM and MH), and a grant from the Caisse Centrale de la Mutualité Sociale
468 Agricole (to MH). Additional support came from an NSERC Discovery to GJM. The funders
469 had no role in the study design, data collection and analyses, decision to publish or preparation
470 of the manuscript. The authors report no conflicts of interest. We thanks two anonymous
471 referees for their comments on the manuscript.

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473 7. REFERENCES

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732 TABLES

733 **Table 1. Diagnostic symptoms of human clinical depression (major depressive disorder)**
 734 **in the Diagnostic Manual of Mental Disorders DSM- fifth edition (APA, 2013, pp. 160-**
 735 **161).**

736 The DSM is a widely-used diagnostic guide in human psychological medicine and potentially
 737 useful for application to non-human animals (Ferdowsian et al., 2011). Approximately every
 738 two decades it is revised, reflecting the challenges of identifying forms of illness that are
 739 complex and sometimes have no single defining criterion. However, since the DSM-III (APA,
 740 1980) its criteria for depression have been essentially stable, with anhedonia playing a central
 741 role. Currently, “five (or more) of the following symptoms have been present during the same
 742 2-week period and represent a change from previous functioning; at least one of the symptoms
 743 is either (1) depressed mood or (2) loss of interest or pleasure” (DSM-V p160) (§ *in the table*).
 744 These symptoms must also not be due to another medical condition, *e.g.* not better explained
 745 by schizophrenia or other psychotic disorders.

746 (*) represent symptoms we consider not reliably or validly operationalisable in non-human animals.

#	Symptom description
1 (§)	Depressed mood most of the day, nearly every day, as indicated by either subjective report (<i>e.g.</i> feels sad, empty, hopeless) or observation made by others (<i>e.g.</i> appears tearful). (<i>Note:</i> in children or adolescents, this can be manifest as irritability.)
2 (§)	Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation)

3 Significant weight loss, despite not dieting, or weight gain (*e.g.* a change of more than 5% of body weight in a month), or a decrease or increase in appetite nearly every day. (*Note:* in growing children, this may be manifest as failures to make expected weight gain.)

4 Insomnia or hypersomnia nearly every day.

Psychomotor agitation or retardation nearly every day (observable by others: not merely subjective feelings of restlessness or being slowed down).

5 (*) Common manifestations of *psychomotor retardation* include “slow speech, thinking and body movements; increased pauses before answering; speech that is decreased in volume, inflection, amount, variety of content, or muteness”; and of *psychomotor agitation*: “the inability to sit still, pacing, hand-wringing, pulling or rubbing of the skin, clothing, or other objects” (DSM-V, p163)

6 Fatigue or loss of energy nearly every day.

7 (*) Feelings of worthlessness or excessive or inappropriate guilt nearly every day (which may be delusional; and are beyond mere self-reproach or guilt about being sick).

8 Diminished ability to think or concentrate, or indecisiveness, nearly every day (assessed either *via* subjective self-report or as observed by others).

9 (*) Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

748 **Table 2: subjects' characteristics: age, sex and whether they previously have been (or**
749 **not) studied in the Fureix's et al (2012) study**

750

Horse ID	Age (years)	Sex	Previously studied?
1	20	Female	Yes
2	18	Gelding	Yes
3	17	Female	Yes
4	16	Gelding	Yes
5	16	Gelding	Yes
6	15	Gelding	Yes
7	15	Gelding	Yes
8	14	Gelding	Yes
9	18	Gelding	Yes
10	18	Gelding	Yes
11	17	Gelding	Yes
12	17	Gelding	Yes
13	16	Gelding	Yes
14	14	Gelding	Yes
15	13	Gelding	Yes
16	8	Gelding	No
17	15	Female	No
18	7	Gelding	No
19	9	Female	No
20	7	Gelding	No

751

752 **Table 3. Name and description of the stereotypic behaviour recorded (adapted from**
 753 **Hausberger et al., 2009; Fureix et al., 2011a).**

754

Name	Description
Crib-biting	The horse grasps a fixed object with its incisors, pulls back, and draws air into its oesophagus while emitting a characteristic pharyngeal grunt.
Lip play	The horse moves its upper lip up and down without making contact with an object, or the horse smacks its lips together (three movements or more in a bout).
Tongue play	The horse sticks out its tongue and twists it in the air (three movements or more in a bout).
Lip or teeth rubbing	The horse rubs its upper lip or its upper teeth repetitively against the box wall (three movements or more in a bout).
Weaving	The horse sways laterally, moving its head, neck, forequarters, and sometimes hindquarters (three movements or more in a bout).
Head shaking and nodding	The horse bobs repetitively its head up and down or tosses its head in recurrent and sudden bouts (three movements or more in a bout).

755

756 FIGURE CAPTIONS

757 **Figure 1: The posture of “withdrawn” horses.** Pictures of a horse a) in a withdrawn posture,
758 b) standing observing and c) standing resting. The withdrawn state is characterized by a
759 stretched neck (obtuse jaw-neck angle) and a similar height between the horse’s neck and back
760 (a nape–withers–back angle of $\approx 180^\circ$). This posture is different from those associated with
761 observation of the environment (for which the neck is higher), and resting, when eyes are at
762 least partly closed and the horse’s neck is rounder (Waring, 2003; Fureix et al., 2011b). Note
763 that the restricted size of the stall (3m x 3m) prevented the authors from taking a picture of the
764 whole horse displaying the withdrawn posture, as we chose to use the same lens to limit shape
765 distortion between images.

766

767 **Figure 2. Relationship between the time spent being withdrawn and sucrose intake,**
768 **controlling for age, sex and time spent in the stall (thus able to eat the sugar) over testing.**
769 Black diamonds: after 3h of exposure; dark grey squares: after 8h of exposure; lighter grey
770 triangles: after 24h of exposure; lightest grey crosses: after 30h of exposure.

771

772 **Figure 3. Relationship between the time spent being withdrawn and sucrose intake,**
773 **controlling for age, sex and time spent in the stall, hay consumption and latency to eat a**
774 **meal of normal pellets scented with novel aromatics.** Black diamonds: after 3h of exposure;
775 dark grey squares: after 8h of exposure; lighter grey triangles: after 24h of exposure; lightest
776 grey crosses: after 30h of exposure.

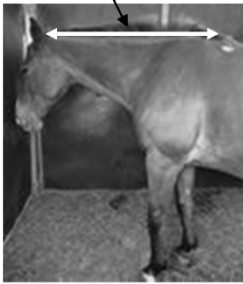
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Angle nape - withers - back = 180°

Closed or partly closed eyes



a) "withdrawn"

b) standing observing

c) standing resting

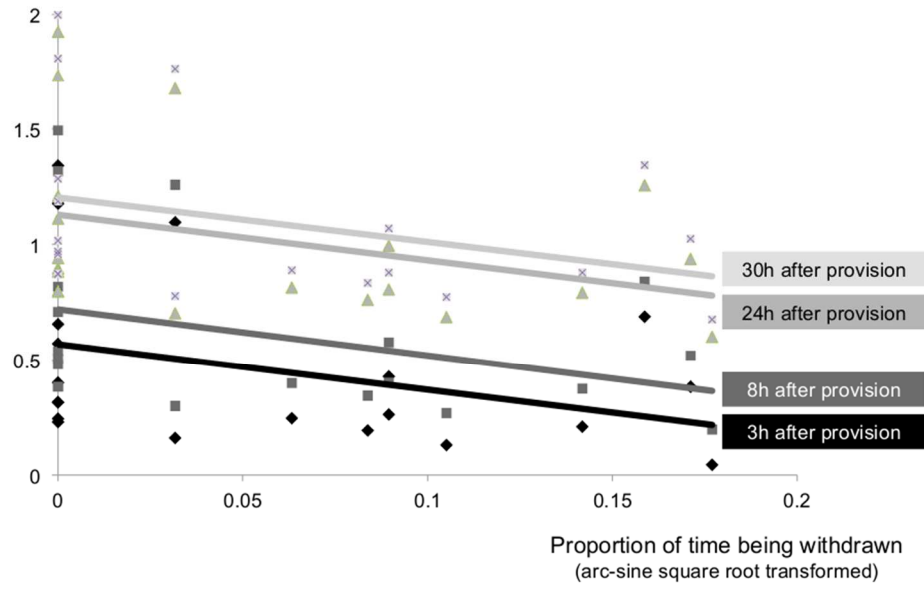
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Fig1

Least square mean proportion of sugar the horse ate out of the 600g (arc-sine square root transformed)



783

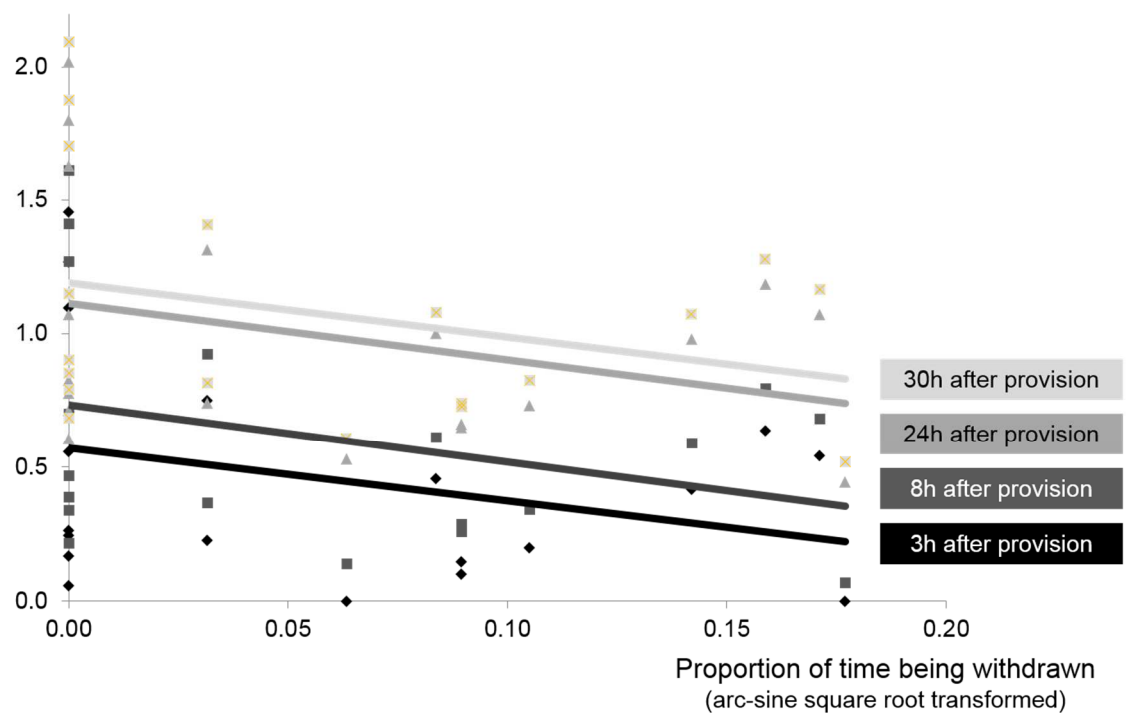
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Fig2

Least square mean proportion of sugar the horse ate out of the 600g
(arc-sine square root transformed)



787

788

789

Fig3