

1 **Investigating attentional processes in depressive-like**
2 **domestic horses (*Equus caballus*).**
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38 **Abstract**

39 Some captive/domestic animals respond to confinement by becoming inactive and
40 unresponsive to external stimuli. Human inactivity is one of the behavioural markers of
41 clinical depression, a mental disorder diagnosed by the co-occurrence of symptoms including
42 deficit in selective attention. Some riding horses display ‘withdrawn’ states of inactivity and
43 low responsiveness to stimuli that resemble the reduced engagement with their environment
44 of some depressed patients. We hypothesized that ‘withdrawn’ horses experience a
45 depressive-like state and evaluated their level of attention by confronting them with auditory
46 stimuli. Five novel auditory stimuli were broadcasted to 27 horses, including 12 ‘withdrawn’
47 horses, for 5 days. The horses’ reactions and durations of attention were recorded. Non-
48 withdrawn horses reacted more and their attention lasted longer than that of withdrawn horses
49 on the first day, but their durations of attention decreased over days, but those of withdrawn
50 horses remained stable. These results suggest that the withdrawn horses’ selective attention is
51 altered, adding to already evidenced common features between this horses’ state and human
52 depression.

53 Key words: horses; attention; cognition; welfare; depression;

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65 **1. Introduction**

66 One of captive/domestic animals' responses to confinement is to become inactive and
67 unresponsive to external stimuli (Fuchs & Flügge, 2002), as reported for many lab/farm
68 species experiencing inappropriate living conditions (*e.g.* fur-farmed mink; tethered sows;
69 caged dogs, Cronin, 1985). Humans' inactivity is one of the behavioural markers of clinical
70 depression (APA, 2013), a complex heterogeneous mental disorder diagnosed by the co-
71 occurrence of affective, cognitive and behavioural symptoms (APA, 2013). Symptoms
72 include deficits in selective attention (*i.e.* ability to focus perception on one stimulus while
73 filtering out other simultaneous stimuli, Posner *et al.*, 1980), as evidenced *e.g.* by slower
74 reactions in an auditory oddball task (Kemp *et al.*, 2010). Most reports of horses' impaired
75 welfare (Burn *et al.*, 2010; Pritchard *et al.*, 2005) or pain (*e.g.* Ashley *et al.*, 2005; review in
76 Hausberger *et al.*, in press) indicate that some horses are unresponsive to environmental
77 stimuli. A 'withdrawn' state was recently described more formally (Fureix *et al.*, 2012):
78 during a withdrawn bout, horses remain totally motionless (immobility with no neck, head,
79 and ear movements); displaying a flat-necked posture; wide open, rarely blinking eyes; and
80 backwards-pointing ears. Compared to non-withdrawn horses from the same stable,
81 withdrawn horses react less to human approach and tactile stimulation, and consume less
82 sucrose (Fureix *et al.*, 2015), a sign of anhedonia *i.e.* loss of interest or pleasure (Willner *et*
83 *al.*, 1992), which is a core symptom of human clinical depression. We hypothesized that if
84 withdrawn horses were in a depression-like condition their selective attention would be
85 altered (Greimel *et al.*, 2015). Therefore we submitted 12 withdrawn horses and 15 non-
86 withdrawn control horses from the same stable to unfamiliar auditory stimuli.

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88 **2. Material and Methods**

89 *2.1. Ethical note*

90 This study complied with French laws related to animal experimentation and the
91 European directive 86/609/CEE.

92

93 *2.2. Subjects*

94 Twenty-seven horses ($N_{\text{mares}}=6$; $N_{\text{geldings}}=21$; aged 5-20 years, $\bar{X} \pm \text{SE}=12.7 \pm 0.9$, 77% French
95 Saddlebred) from the same riding school were observed in June 2012. The horses were kept in
96 3m * 3m individual straw-bedded stalls in a barn, allowing visual contact with conspecifics.

97 Each stall was cleaned every morning, and was equipped with an automatic drinker. Animals
98 were fed hay once a day (13:00 h), and commercial pellets three times a day (07:00 h, 12:00
99 h, 19:00 h). Groups of 7-8 horses were released (allowing contact) into barren paddocks for
100 two days at the week-end. Horses worked in riding lessons for 6–10 hours a week (two rest
101 days). The time each horse spent being withdrawn in its stall was determined using
102 instantaneous scan sampling (Altmann, 1974) every 2 minutes for 1h periods, repeated daily
103 at different times of day (from 6:00 a.m. to 9:00 p.m.) over 15 days (average number of total
104 scans per subject: 907 ± 11.14) (Fureix *et al.*, 2015). Twelve of the 27 horses ($N_{\text{mares}}=3$;
105 $N_{\text{geldings}}=9$; called withdrawn horses hereafter) displayed the previously described withdrawn
106 state at least once.

107

108 2.3. Test settings

109 Five stimuli, all initially novel for the subjects, were broadcasted within the stable: three
110 vocalizations of different species (baboon, barnacle goose and whale), one vocalization of an
111 unfamiliar conspecific, and one non-biological sound (piano music). Horses were randomly
112 exposed by groups of 4-6 in neighbouring stalls, following Noble *et al.*, (2013), to one of the
113 five auditory stimuli, which differed for each group on a given day (*e.g.* the 1st test day, the 1st
114 group heard the baboon, the 2nd the whale etc; the 2nd test day the 1st group heard the piano,
115 the 2nd the baboon etc). The loudspeaker (Nagra Kudelski HP monitor®) was at equal
116 distances (2 m) from each horse. Stimuli were broadcast for 3 seconds at 80db, always
117 between 13:00h and 14:00h and for 5 consecutive days. Data collected on the fifth test day
118 were discarded, due to a different (from the other test days) horses' management (*i.e.* no
119 riding activities). Proportions of non-withdrawn and withdrawn horses were balanced within
120 each group. Observations focused on small groups because we could not isolate subjects, or
121 observe all the 27 horses simultaneously in the barn.

122

123 2.4. Behavioural measurements

124 Tests were videotaped (Sony HDR-XR105®) and standard measures of attentional states
125 were extracted from videos using continuous focal sampling (Altmann, 1974). We recorded
126 the presence or absence of reaction (*i.e.* change in behaviour interrupting the ongoing activity
127 after a broadcast, characterized by any ear, head, neck or whole body movement) to estimate
128 attentional capture. We recorded the total duration of attention (*i.e.* standing motionless with

129 eyes, ears, head or neck oriented towards the loudspeaker (Waring, 2003)) during the 5
130 minutes following a broadcast. Three horses (1 non-withdrawn, 2 withdrawn) were scared by
131 the stimuli on the 1st and 2nd test days (*i.e.* alarm posture associated with dilated nostrils and
132 snorting and active walking in the box; Kiley-Worthington, 1976, Wolff *et al.*, 1997). As
133 these horses did not show any attention patterns, they were excluded from the analyses, so our
134 analyses included only 24 horses.

135

136 *2.5. Statistical analyses*

137 Normality and homogeneity of variances were assessed by inspection of residuals and
138 Shapiro–Wilk W tests (Ha and Ha, 2011). Because our data were not normally distributed, we
139 applied non-parametric statistical tests (Siegel and Castellan, 1988). Fisher’s exact and Chi-
140 square tests were used to evaluate the influence of test day on the proportion of reactive
141 *versus* non-reactive horses. Friedman tests, followed by multiple pairwise comparisons using
142 Wilcoxon signed rank t-tests applying a False discovery rate correction (Benjamini and
143 Hochberg, 2000) were used to evaluate relationships between duration of attention, stimulus
144 type and test day. Kruskal-Wallis tests were used to assess relationships between duration of
145 attention and group observation. Mann-Whitney U-tests (for each test day) compared
146 durations of attention between non-withdrawn (NW) and withdrawn (W) horses, and
147 gelding/mares. Analyses were conducted using R software (accepted two-tailed p level at
148 0.05). Descriptive statistics are means (\bar{X}) followed by standard errors (SE).

149

150 **3. Results**

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152 Neither sex nor type of stimulus nor observation group influenced reactions and durations
153 of attention significantly (*sex*: Mann-Whitney U-tests, $P > 0.05$; *stimuli*: Friedman tests,
154 $1.2 < F < 7.2$, $P > 0.05$; *group*: Kruskal-Wallis tests, $6.8 < H < 10.2$, $P > 0.05$), therefore data were
155 pooled for subsequent analyses.

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157 *3.1. First test day*

158 More NW horses (93%) than W horses (50%) reacted (Fisher exact Test, $P=0.02$) (Fig. 1).
159 Reaction times ranged from 0.41 to 4.48 seconds ($\bar{X}=1.8\pm0.2$ seconds), but did not differ
160 between NW and W horses (Mann-Whitney U-test: $N_{NW}=14$, $N_W=10$, $\bar{X}_{NW}=2.0\pm0.2s$,
161 $\bar{X}_W=1.2\pm0.2s$; $U=28$, $P=0.27$).

162 Durations of attention in reactive horses varied between subjects (from 1.0 to 10.8
163 seconds, $\bar{X}=4.2\pm0.7$) and were longer for NW horses than for W horses (Mann-Whitney U-
164 test: $N_{NW}=14$, $N_W=10$, $\bar{X}_{NW}=5.6\pm0.8s$, $\bar{X}_W=2.4\pm1.1s$; $U=38$, $P=0.01$) (Fig. 2).

165

166 3.2. Changes of response to auditory stimuli in relation to time

167 Although all horses paid attention to the auditory stimuli at least once, patterns of
168 response of W and NW horses differed in relation to time. The proportion of reactive W
169 horses increased significantly on day 2 ($X^2=10.5$ $p=0.0002$) and remained high on the
170 following days, contrary to NW horses (Fig. 1). Durations of attention of NW horses
171 decreased on day 2 compared to day 1 (Friedman test ($N=14$, $df=4$)= 12.4 $p=0.01$; Wilcoxon
172 signed-rank test $\bar{X}_{D1}=5.6\pm10.8s$, $\bar{X}_{D2}=2.9\pm0.6s$, $\bar{X}_{D3}=3.1\pm10.7$, $\bar{X}_{D4}=3.7\pm0.5$, $p<0.05$
173 for all), whereas they did not vary significantly for W horses (Friedman test ($N=10$, $df=4$)= 1.3
174 $p=0.85$) (Fig. 2).

175

176 4. Discussion

177 Playback of unfamiliar auditory stimuli elicited reactions of non-withdrawn horses but of
178 only half of the withdrawn horses on the first day. Durations of attention of reactive non-
179 withdrawn horses were longer than those of reactive withdrawn horses. Non-withdrawn
180 horses' durations of attention decreased on the following days, but those of withdrawn horses
181 did not vary significantly.

182 On the first test day, withdrawn horses seemed to have "switched off" auditory stimuli
183 compared to non-withdrawn horses. This result is in accordance with Fureix *et al.*'s (2012)
184 study reporting withdrawn horses' lower levels of reactions towards humans and tactile
185 stimuli. This "switch off" might reflect the withdrawn horses' lack of selective attention
186 towards unusual stimuli. Several hypotheses have been proposed to explain the depressed

187 human patients' lowered selective attention: 1) negative emotional states draw attention and
188 thus interrupt associated behaviour: the more intensive a negative emotional state, the more it
189 can disrupt attention to anything else (Smith et al., 1985; Williams, 2002); and 2) the resource
190 allocation hypothesis postulates that because the depressed patients' cognitive capacity is
191 reduced, they have deficits in engaging in effortful cognitive processes such as focusing on
192 relevant stimulations (Ellis & Ashbrook, 1988, cited by Gotlib & Joormann, 2010). In this
193 study, both hypotheses may apply. The complete immobility, the backward ears and lack of
194 eye blinking (Fureix *et al.*, 2012) argue in favour of inward oriented attention more than
195 attention oriented towards the environment. This hypothesis is confirmed by our data on
196 attention for novel auditory stimuli. Thus, horses with impaired welfare may not perform
197 cognitive tasks well (Hausberger *et al.*, 2007).

198 With time, the subjects' reactions to our tests varied. More withdrawn horses reacted on
199 the second day and this proportion remained similar subsequently, while a reverse pattern was
200 observed for the non-withdrawn horses. Withdrawn horses could have difficulties to detect or
201 differentiate novel or relevant stimuli in their environment, and this could be related to
202 cognitive control deficits affecting individuals' ability to disengage attention from irrelevant
203 material, a common feature of human depressed patients (Gotlib and Joormann, 2010; Kemp
204 *et al.*, 2010).

205
206 This study reveals that a horses' 'withdrawn state' corresponds to a multifaceted
207 syndrome including lower reactivity to environmental stimuli suggesting selective attentional
208 impairments, a common trait of human depressed patients (Gotlib and Joormann, 2010).
209 Replication and further research on withdrawn horses' attentional processes are needed,
210 before validating it for a spontaneous model of depression.

211

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222 **References**

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224 Altmann, J., 1974. Observational Study of Behavior: Sampling Methods. *Behaviour* 49, 227–
225 266. doi:10.1163/156853974X00534

226 APA, A.P., 2013. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.:
227 DSM 5. bookpointUS.

228 Ashley, F.H., Waterman-Pearson, A.E., Whay, H.R., 2005. Behavioural assessment of pain in
229 horses and donkeys: application to clinical practice and future studies. *Equine Vet. J.*
230 37, 565–575. doi:10.2746/042516405775314826

231 Benjamini, Y., Hochberg, Y., 2000. On the Adaptive Control of the False Discovery Rate in
232 Multiple Testing With Independent Statistics. *J. Educ. Behav. Stat.* 25, 60–83.
233 doi:10.3102/10769986025001060

234 Burn, C.C., Dennison, T.L., Whay, H.R., 2010. Relationships between behaviour and health
235 in working horses, donkeys, and mules in developing countries. *Appl. Anim. Behav.*
236 *Sci.* 126, 109–118. doi:10.1016/j.applanim.2010.06.007

237 Fuchs, E., Flügge, G., 2002. Social stress in tree shrews: Effects on physiology, brain
238 function, and behavior of subordinate individuals. *Pharmacol. Biochem. Behav.* 73,
239 247–258. doi:10.1016/S0091-3057(02)00795-5

240 Fureix, C., Beaulieu, C., Argaud, S., Rochais, C., Quinton, M., Henry, S., Hausberger, M.,
241 Mason, G., 2015. Investigating anhedonia in a non-conventional species: Do some
242 riding horses *Equus caballus* display symptoms of depression? *Appl. Anim. Behav.*
243 *Sci.* 162, 26–36. doi:10.1016/j.applanim.2014.11.007

244 Gotlib, I.H., Joormann, J., 2010. Cognition and Depression: Current Status and Future
245 Directions. *Annu. Rev. Clin. Psychol.* 6, 285–312.
246 doi:10.1146/annurev.clinpsy.121208.131305

247 Greimel, E., Trinkl, M., Bartling, J., Bakos, S., Grossheinrich, N., Schulte-Körne, G., 2015.
248 Auditory selective attention in adolescents with major depression: An event-related
249 potential study. *J. Affect. Disord.* 172, 445–452. doi:10.1016/j.jad.2014.10.022

250 Ha, R.R., Ha, J.C., 2011. *Integrative Statistics for the Social and Behavioral Sciences.* SAGE.

251 Hausberger, M., Gautier, E., Müller, C., Jego, P., 2007. Lower learning abilities in stereotypic
252 horses. *Appl. Anim. Behav. Sci.* 107, 299–306. doi:10.1016/j.applanim.2006.10.003

253 Hausberger, M., Fureix, C., Lesimple, C., in press. Detecting horses' sickness: in search of
254 visible signs.

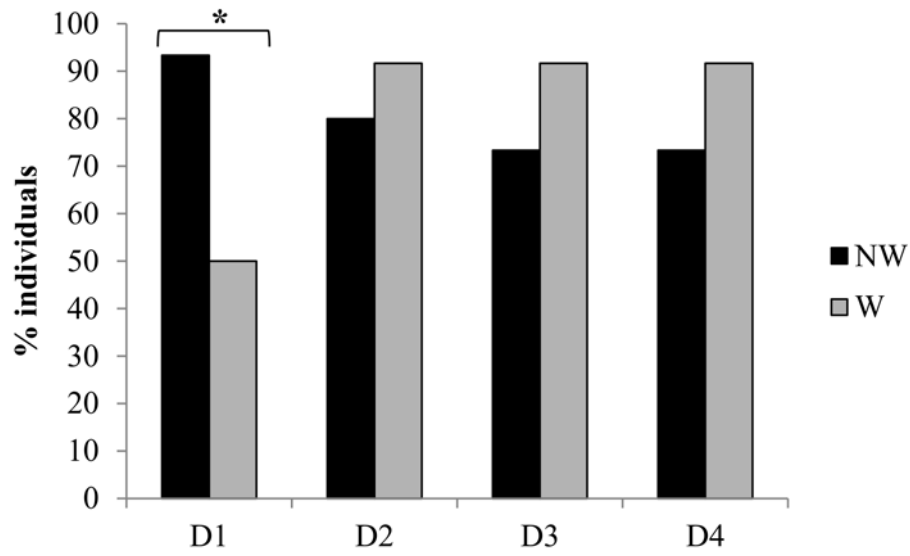
255 Kemp, A.H., Pe Benito, L., Quintana, D.S., Clark, C.R., McFarlane, A., Mayur, P., Harris, A.,
256 Boyce, P., Williams, L.M., 2010. Impact of depression heterogeneity on attention: An
257 auditory oddball event related potential study. *J. Affect. Disord.* 123, 202–207.
258 doi:10.1016/j.jad.2009.08.010

259 Kiley-Worthington, M., 1976. The tail movements of ungulates, canids and felids with
260 particular reference to their causation and function as displays. *Behaviour* 56, 69–115.
261 doi:10.1163/156853976X00307

262 Noble, G.K., Blackshaw, K.L., Cowling, A., Harris, P.A., Sillence, M.N., 2013. An objective
263 measure of reactive behaviour in horses. *Appl. Anim. Behav. Sci.* 144, 121–129.
264 doi:10.1016/j.applanim.2012.12.009

- 265 Posner, M.I., Snyder, C.R., Davidson, B.J., 1980. Attention and the detection of signals. *J.*
266 *Exp. Psychol. Gen.* 109, 160–174. doi:10.1037/0096-3445.109.2.160
- 267 Pritchard, J.C., Lindberg, A.C., Main, D.C.J., Whay, H.R., 2005. Assessment of the welfare of
268 working horses, mules and donkeys, using health and behaviour parameters. *Prev. Vet.*
269 *Med.* 69, 265–283. doi:10.1016/j.prevetmed.2005.02.002
- 270 Siegel, S., Castellan, N.J., 1988. *Nonparametric statistics for the behavioral sciences.*
271 McGraw-Hill, New York.
- 272 Smith, D.T.W., Ingram, R.E., Roth, D.L., 1985. Self-focused attention and depression: Self-
273 evaluation, affect, and life stress. *Motiv. Emot.* 9, 381–389. doi:10.1007/BF00992207
- 274 Waring, G.H., 2003. *Horse Behavior.* Noyes Publications/William Andrew Pub.
- 275 Williams, A.C. de C., 2002. Facial expression of pain, empathy, evolution, and social
276 learning. *Behav. Brain Sci.* 475.
- 277 Willner, P., Muscat, R., Papp, M., 1992. Chronic mild stress-induced anhedonia: A realistic
278 animal model of depression. *Neurosci. Biobehav. Rev.* 16, 525–534.
279 doi:10.1016/S0149-7634(05)80194-0
- 280 Wolff, A., Hausberger, M., Le Scolan, N., 1997. Experimental tests to assess emotionality in
281 horses. *Behav. Processes* 40, 209–221. doi:10.1016/S0376-6357(97)00784-5
282

283

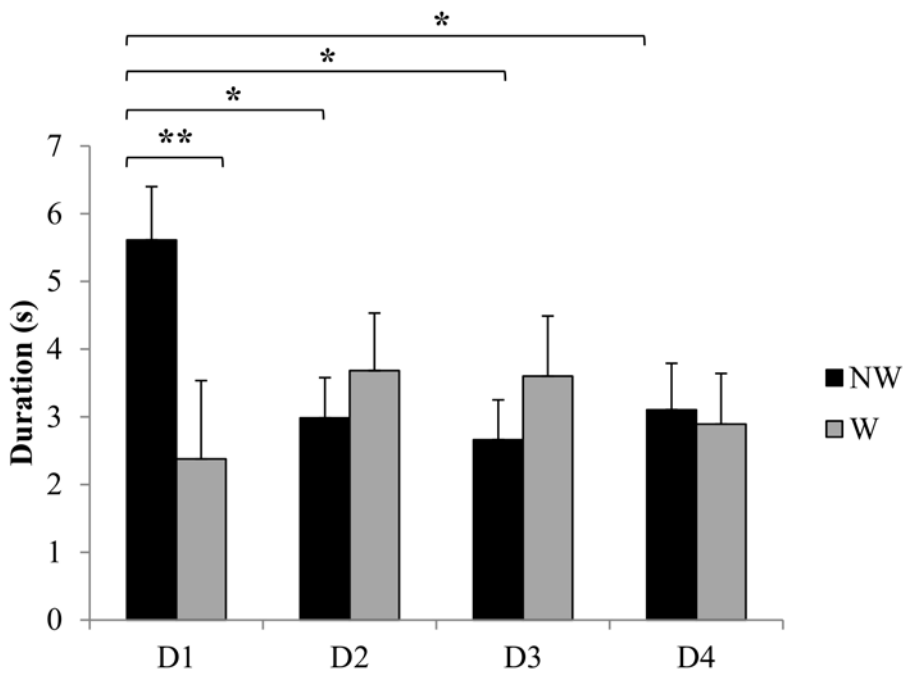


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Figure 1

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Figure 2

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