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 Some riding horses display 'withdrawn' states of inactivity and 42 low responsiveness to stimuli that resemble the reduced engagement with their environment 43 of some depressed patients. We hypothesized that 'withdrawn' horses experience a 44 depressive-like state and evaluated their level of attention by confronting them with auditory 45 stimuli. Five novel auditory stimuli were broadcasted to 27 horses, including 12 'withdrawn' 46 horses, for 5 days.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 Non-47 withdrawn horses reacted more and their attention lasted longer than that of withdrawn horses 48 on the first day, but their durations of attention decreased over days, but those of withdrawn 49 horses remained stable. These results suggest that the withdrawn horses' selective attention is 50 altered, adding to already evidenced common features between this horses' state and human 51 depression. 52

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

65 **1. Introduction**

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 A 'withdrawn' state was recently described more formally (Fureix et al., 2012): 77 78 during a withdrawn bout, horses remain totally motionless (immobility with no neck, head, and ear movements); displaying a flat-necked posture; wide open, rarely blinking eyes;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

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- 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.

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93 2.2. Subjects

Twenty-seven horses (N_{mares}=6; N_{geldings}=21; aged 5-20 years, $\overline{X} \pm SE=12.7\pm0.9$, 77% French Saddlebred) from the same riding school were observed in June 2012. The horses were kept in 3m * 3m individual straw-bedded stalls in a barn, allowing visual contact with conspecifics.

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

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108 2.3. Test settings

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

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2.4. Behavioural measurements

Tests were videotaped (Sony HDR-XR105®) and standard measures of attentional states were extracted from videos using continuous focal sampling (Altmann, 1974). We recorded the presence or absence of reaction (*i.e.* change in behaviour interrupting the ongoing activity after a broadcast, characterized by any ear, head, neck or whole body movement) to estimate attentional capture. We recorded the total duration of attention (*i.e.* standing motionless with eyes, ears, head or neck oriented towards the loudspeaker (Waring, 2003)) during the 5 minutes following a broadcast. Three horses (1 non-withdrawn, 2 withdrawn) were scared by the stimuli on the 1st and 2nd test days (*i.e.* alarm posture associated with dilated nostrils and snorting and active walking in the box; Kiley-Worthington, 1976, Wolff *et al.*, 1997). As these horses did not show any attention patterns, they were excluded from the analyses, so our analyses included only 24 horses.

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136 *2.5.Statistical analyses*

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

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150 **3. Results**

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

More NW horses (93%) than W horses (50%) reacted (Fisher exact Test, P=0.02) (Fig. 1). Reaction times ranged from 0.41 to 4.48 seconds ($\overline{X} = 1.8 \pm 0.2$ seconds), but did not differ between NW and W horses (Mann-Whitney U-test: N_{NW}= 14, N_W= 10, $\overline{X}_{NW} = 2.0 \pm 0.2s$, $\overline{X}_{W} = 1.2 \pm 0.2s$; U = 28, P= 0.27).

Durations of attention in reactive horses varied between subjects (from 1.0 to 10.8 seconds, $\overline{X} = 4.2 \pm 0.7$) and were longer for NW horses than for W horses (Mann–Whitney Utest: N_{NW}= 14, N_W= 10, \overline{X} _{NW}= 5.6 ± 0.8s, \overline{X} _W= 2.4 ± 1.1s; U = 38, P= 0.01) (Fig. 2).

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

Although all horses paid attention to the auditory stimuli at least once, patterns of 167 response of W and NW horses differed in relation to time. The proportion of reactive W 168 horses increased significantly on day 2 ($X^2=10.5$ p=0.0002) and remained high on the 169 following days, contrary to NW horses (Fig. 1). Durations of attention of NW horses 170 decreased on day 2 compared to day 1 (Friedman test (N=14, df=4)=12.4 p=0.01; Wilcoxon 171 signed-rank test $\overline{X}_{D1} = 5.6 \pm 10.8$ s, $\overline{X}_{D2} = 2.9 \pm 0.6$ s, $\overline{X}_{D3} = 3.1 \pm 10.7$, $\overline{X}_{D4} = 3.7 \pm 0.5$, p<0.05 172 for all), whereas they did not vary significantly for W horses (Friedman test (N=10, df=4)=1.3 173 174 p=0.85) (Fig. 2).

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176 **4. Discussion**

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

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

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

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

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

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212 Acknowledgments

We would like to thank the managers of the riding school for allowing us to work with their horses and all staff for their help and cooperation. This work was supported by a doctoral grant from the French Research Minister (CR), a Fyssen foundation postdoctoral fellowship (CF), a grant from the French Canada Research Fund, and from the Institut Français du Cheval et de l'Equitation (IFCE). The project benefitted from discussions from the GIS CCS. The funders had no role in the study design, data collection and analysis, decision to publish

- 219 or preparation of the manuscript. The authors report no conflicts of interest. We thank the
- anonymous referees for their comments on the manuscript.

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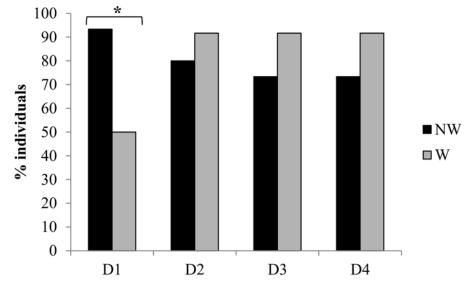




Figure 1

