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

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ABSTRACT

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 Some riding horses display 'withdrawn' states of inactivity and low responsiveness that resemble the reduced engagement with the environment shown by certain depressed patients.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a depression-like state, we investigated anhedonia -- the loss of pleasure, a key symptom of human depression -- in 20 withdrawn and non-withdrawn horses from the same stable. The time horses spent being withdrawn appeared unrelated to age or sex, but correlated with time devoted to stereotypic behaviour, a possible marker of lifetime stress. Comparison with data collected 5 years earlier also revealed that horses scored as withdrawn then remained significantly likely to display the behaviour.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 We predicted that if affected by depression-like states, the most withdrawn horses would consume the least sucrose.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low sucrose consumption, statistically controlling for these confounds did not eliminate the relationship between being withdrawn and consuming less sucrose (although reducing it to a strong trend): $F_{1,15} = 4.28$, two-tailed $p = 0.056$.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in certain riding horses, which correlate with stereotypic behaviour and are characterised by anhedonia and bouts of 'withdrawn' unresponsiveness.

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

1. INTRODUCTION

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

In terms of aetiology, a common trigger is chronic stress, for instance that arising from aversive life events or chronic pain or illness (*e.g.* Banks and Kerns, 1996; Blackburn-Munro and Blackburn-Munro, 2001; Tafet and Bernardini, 2003; Munce et al., 2006; Siegrist, 2008; Hammen et al., 2009; APA, 2013). Two types of cognitive change can often be observed before the illness fully develops, and these may act as mediators in some subjects, being hypothesised to help cause the onset and/or maintenance of the disease (Beck, 1967; Gotlib and Krasnoperova, 1998). One is 'learned helplessness', which is proposed to occur "when highly desired outcomes are believed improbable or highly aversive outcomes are believed probable, and the individual comes to expect that no response in his repertoire will change their likelihood" (Abramson et al., 1978). The second involves negative biases in attention, memory and/or judgment (Beck, 1967; MacLeod and Byrne, 1996; Gotlib and Krasnoperova, 1998). Thus depressed people are prone for example, to judge ambiguous stimuli as being unlikely to be positive ('cognitive pessimism'), and to recall unpleasant memories more readily than pleasant ones.

The symptoms of depression may not be unique to humans. A Web of Science literature search using the terms "rats" OR "mice" OR "monkeys" AND "depression" yielded over 100,000 articles: laboratory rodents in particular are widely used to model clinical depression, primarily to screen drugs for human use (reviewed in [e.g.] Matthews et al., 2005; Deussing, 2006). Although "it is exceedingly difficult to envision an animal model that perfectly recapitulates the symptoms of depression in human patients" (Deussing, 2006), much of this animal work does appear to validly model at least some symptoms of this disease. For an animal condition to be deemed homologous to a human illness, it must display several forms of validity (Belzung and Lemoine, 2011; Camus et al., 2013a): symptoms should seem analogous to those of affected humans (sometimes called "face validity"); the animal's condition should mimic the human disease in terms of risk, protective and therapeutic factors (sometimes called "predictive validity"); and underlying mechanisms should ideally be homologous (although for depression, this would require a consensus about its mechanistic bases that does not currently exist, see [e.g.] Moore, 2002; Strickland et al., 2002). By exposing laboratory animals to chronic unpredictable stress – as well as to lesions, drugs and genetic manipulations that arguably have less predictive validity (Deussing, 2006) – researchers have successfully induced responses that have face validity with certain features of human depression: "helpless" reactions to unavoidable stressors (e.g. Maier and Seligman, 1976), along with anhedonia (e.g. Willner et al., 1992), a reduction in pleasure that we discuss in detail below.

Furthermore, outside of this type of research environment, circumstantial evidence has led several authors to hypothesise that depression-like states occur in other animals. Pet dogs and cats have been suggested to show "depressed behaviour" when deprived of their owners (Fox, 1968, p. 357), as have apes housed long-term in barren environments in laboratories or zoos (e.g. Engel, 2002 p174, Brune et al., 2006; Ferdowsian et al., 2011; Hennessy et al., 2014) and maternally deprived monkeys (e.g. Harlow and Suomi, 1974; Suomi et al., 1975; Hennessy et al., 2014). Horses, too, have been suggested to sometimes display depression-like symptoms (Pritchard et al., 2005; Hall et al., 2008; Burn et al., 2010; Fureix et al., 2012; Popescu and Diugan, 2013). States involving profound inactivity and low responsiveness to external stimuli have thus been reported in working equids in the developing world (Swann, 2006; Burn et al., 2010), and in riding horses in Europe and North America (Fureix et al., 2012). For example, Hall and colleagues describe riding school horses "who seem to have 'switched off' (are unresponsive, lack motivation, and are apathetic)". Recently, data have been collected to describe such horses' characteristics more formally. Fureix et al. (2012) found that 'withdrawn' states are characterised by a stationary, atypical, flat-necked posture (**Figure 1**); wide open, unblinking eyes with an apparently fixed gaze; and backwards-pointing ears. Even when not engaged in these unusual behaviours, horses with these states differed from non-withdrawn horses from the same stable in several ways: they show reduced responsiveness to tactile stimulation; less reaction to a human's sudden appearance at the stall door; less exploration and more behavioural signs of arousal (fear) when exposed to a novel object; and lower baseline levels of plasma cortisol. Furthermore, while the full aetiology of such states is still unclear, being withdrawn was more prevalent in female riding horses than male (Fureix et al., 2012); inactivity combined with low responsiveness to external stimuli is, in working equids, associated with illness and advanced age (Burn et al., 2010); and, more speculatively, sustained adversity has been hypothesised to play a role. Thus Hall et al. (2008) suggested that "apathetic and lethargic" horses have generally been "stabled for the majority of their lives with no opportunity for social interaction", with such responses reflecting stress-induced "learned helplessness" (see also Ödberg, 1987). Fureix et al. (2012) therefore hypothesised, by analogy with human clinical depression, that withdrawn horses exhibit a depression-like state.

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

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

This work thus aimed to use this measure to test the hypothesis that withdrawn horses are experiencing a depression-like condition. Using withdrawn and non-withdrawn horses from the same riding stable, we predicted that, if in depression-like states, withdrawn horses would consume less sucrose than non-withdrawn stable-mates. To both refine this experiment and better characterise the withdrawn state, we also assessed some additional variables. Most of our subjects had been screened for withdrawn states five years previously (Fureix et al., 2012); we therefore opportunistically investigated whether individuals displaying withdrawn postures in 2007 would still be prone now. In the absence of direct information on the types of aversive early life experience and stressful life events that predispose humans to depression (*e.g.* Gilmer and McKinney, 2003; Tafet and Bernardini, 2003; Siegrist, 2008), we also assessed our subjects' levels of stereotypic behaviour. In populations with diverse past experiences, stereotypic behaviour, although definitely not a symptom of depression, is typically most prevalent or severe in individuals that have experienced challenges during early development and/or stressful lives since (Lutz et al., 2003; Mason and Latham, 2004; Jones et al., 2011; Gottlieb et al., 2013). Assuming this holds for horses, we used stereotypic behaviour as a proxy measure of individuals' life stress, predicting positive relationships between it and the display of withdrawn states. Finally, we checked for three additional confounds that, while not themselves markers of depression, could potentially mediate a positive relationship between withdrawn behaviour and sucrose intake. The first was general appetite at the time of test: checking that general feed consumption levels do not explain sucrose consumption is an important control in rodent and primate biomedical studies of anhedonia (*e.g.* Willner et al., 1992; Paul et al., 2000). The second was food neophobia. The forms of sucrose we used were novel to the subjects (see Methods); withdrawn horses appear to react to novelty more strongly than non-withdrawn horses (Fureix et al., 2012); and in laboratory mice, neophobia increases the latency with which mice ingest solid sugar placed in their cages, independently of anhedonia (Walker and Mason, 2011). We predicted that if in depression-like states, withdrawn horses should still consume less sucrose than non-withdrawn horses even after statistically accounting for these two potential confounds. The third potential confound was withdrawn horses not noticing the offered sucrose because engaged in bouts of non-responsiveness. We therefore assessed sucrose consumption in the first 3 hours after presentation, to check that both withdrawn and non-withdrawn horses sampled it within that short time.

2. METHODS

2.1. Ethical note

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

2.2. Subjects

Twenty horses –16 geldings and 4 mares, aged 7 to 20 years old (on average 14.5 ± 3.9) – were observed between June and November 2012 (**Table 2**). Fifteen had been already studied five years previously (Fureix et al., 2012) (**Table 2**). All came from a single riding school located in western France, at which they had been housed for at least a year. The sample included 17 French Saddlebreds, one French Trotter plus one Anglo-Arabian (two breeds originally involved in the creation of the French Saddlebred breed) and one unregistered horse (thus of unknown breed). Each was kept singly in 3 m x 3 m individual straw-bedded stall in a barn, allowing visual contact with conspecifics. Each stall was cleaned every morning, and was equipped with an automatic drinker. Animals were fed hay (6-7kg) once daily (13:00h), and commercial pellets three times daily (07:00h, 12:00h, 19:00h). These pellets were composed of wheat bran, (30%), barley (28%), alfalfa flour (10%), palm kernels (10%), soya beans (10%), oats (6%), and trace amounts of treacle, corn, calcium carbonate, sodium chloride, vitamins A, D, E and copper sulphate. The horses worked in riding lessons for 4 to 12 hours a week, with two rest days per week during which they were released in groups into paddocks. Riding lessons involved children and teenagers, and both indoor (instruction) and outdoor activities, including a few competitions.

2.3. Behavioural recording: withdrawn status and stereotypic behaviour

The time each horse spent being withdrawn in its stall was determined by a single trained observer, using instantaneous scan sampling (Martin and Bateson, 2007) every 2 minutes over 1h long periods, repeated daily over 15 days. Sampling sessions were conducted during daylight, at 06:00h, 07:00h, 10:00h, 11:00h, 15:00h, 16:00h, 20:00h and 21:00h. The silent observer (CB) walked regularly along the corridor and observations were made at a distance of 3m. The average number of total scans obtained per subject was 907 ± 11.14 (horses were sometimes away from their stall, *e.g.* for lessons, resulting in variation in observation number). All behaviours were recorded (see *e.g.* Waring, 2003 for a detailed ethogram), but we only report here only the states of interest for this study, *i.e.* the time spent being withdrawn, along with stereotypic behaviour (crib-biting, tongue and lips movements, weaving, head shaking and nodding; descriptions in **Table 3**; see also *e.g.* Hausberger et al., 2009; Fureix et al., 2011a). The fully withdrawn state was defined in accordance with Fureix et al. (2012), as follows: the horse was standing motionless, with eyes open but unblinking, without ear or head movements, and displaying a stretched neck (*i.e.* obtuse jaw-neck angle) and a similar height between neck and back (*i.e.* a nape−withers–back angle of 180°) (see also **Fig 1**). A horse displaying all of the above-mentioned characteristics, but blinking the eyes or moving the ears <u>once</u>, was scored as "moderately withdrawn". We chose to do so due to a lack of control over aspects of the environment, such as very windy weather or unpredictable loud noises in the stable, that could potentially very briefly disrupt withdrawn states by inducing a very brief ear movement or eye blinking (therefore violating, although very briefly, the withdrawn definition criteria). Spending time fully withdrawn was extremely strongly related to spending time moderately withdrawn (see Results), supporting *a posteriori* our assumption that the two measures reflected the same state, and we therefore pooled the two measures in the ethogram for further analyses. Note that s*ensu stricto* instantaneous scan sampling was unsuitable for identifying withdrawn states, which were characterised by a lack of movement, and for stereotypic behaviours characterised by repetition. In such instances, the observer switched to focal observation, the activity of the horse of interest being continuously observed for 15 s (see *e.g.* Mason, 1993 and Tilly et al., 2010 for a similar mixture of focal and instantaneous scan sampling).

2.4. Sucrose intake measurement

Over the six days prior to the study, all horses were offered 10g of raw sugar (lumps) once a day, presented by the observer directly under the nostrils, palm up. All horses consumed sugar lumps, revealing no spontaneous aversion towards sucrose in any of the subjects (see *e.g.* Hawkes et al., 1985 for evidence that sucrose consumption is common but not universal in horses). On the first day of the experiment, flavoured sucrose blocks (600g, Likit®, UK), all novel to these horses, were presented under the nostrils of each subject until one lick occurred, and then immediately mounted on the wall of each stall like a salt block. To measure rates of sucrose intake, these sugar blocks were weighed 3h, 8h, 24h and 30h after provision (initial time of block mounting: between 09:00 and 11:00). The 30h long testing period covered both pre- and post-meals intervals, allowing us to control for alliesthesia (modulation of the rewarding value of food according to whether in a deprived or satiated state, Booth et al., 1972; Thompson et al., 1976). To control for possible individual variation in flavour preference (known in horses, Goodwin et al., 2005), the test was replicated three times in succession, using three different Likit® flavours (cherry, banana and apple). Flavour order was pseudo-randomly assigned to control for effects of presentation order.

2.5. Hay consumption and the "novel meal" experiment

All subjects' total hay consumption over a 5 day period was measured to estimate overall food consumption level. Hay nets containing the usual rations were weighed daily pre-feeding (at 13:00h) and after 3h of exposure. Any fallen hay was collected from the bedding and placed back in the hay net prior to each weighing. To assess neophobia towards novel foods, horses' latencies to eat a meal of their usual pellets that had been scented with a mixture of unfamiliar aromatics (10g of aromatic herbs "Herbes de Provence" and 10 ml cl of a solution "Arôme Fleur d'Oranger", Vahiné®) were then measured. Long latencies to interact with a novel object are typically interpreted as reflecting higher levels of anxiety or neophobia (*e.g.* Wolff et al., 1997; Ennaceur et al., 2005). The aromatics and their concentrations were chosen after pilot observations performed on five other horses from the same stable (not study subjects) ensured that the novel flavour in food was perceptible (*e.g.* inducing sniffing) to horses. Meal delivery was performed by the horses' usual caretaker, following the normal daily routine but with an experimenter by her side. The experimenter for this part was SA, not CB, and blind to all horses' results from any other test/observation. Horses were tested one at a time, counterbalanced according to their withdrawn status (*i.e*. testing equal proportions of withdrawn and non-withdrawn horses) across time periods within a day (meal at 07:00h, 12:00h am or 19:00h) and over testing days. The experimenter began to record time immediately after the caretaker poured the pellets into the feeding trough, stopping immediately after the horse took two consecutive mouthfuls without any intervening behaviour other than chewing and swallowing. This caretaker was also blind to the aims of the study, being told that only horses' behaviours were being recorded for further analyses, and asked to behave in her usual way. Each tested horse's latency to eat a normal meal of usual pellets was also recorded (once per horse).

2.6. Statistical analyses

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

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

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

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

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

3. RESULTS

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

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

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

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

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

4. DISCUSSION

As reviewed in the Introduction, our hypothesis that certain horses display depression-like states ($^{e.g.}$ Swann, 2006; Hall et al., 2008; Burn et al., 2010; Fureix et al., 2012) was based on apparent similarities between their reduced responsiveness and the reduced interactiveness of some depressed human patients; evidence of associated anxiety, which would support a diagnosis of depression in humans; hypocortisolaemia, as found in a sub-set of depressed humans; and some preliminary signs of predictive validity (greater occurrence in females and individuals suffering ill-health). To this, we now add evidence of a symptom that has strong face validity with a DSM-V diagnostic criterion, and that has been successfully used in biomedical research on depression-like states in rodents. Assuming that sucrose consumption in horses is driven by pleasure, as in humans and rodents (Jarosz et al., 2006; Lowe and Butryn, 2007; Olsen, 2011; Pratt et al., 2012), then horses that display states of withdrawn reduced reactivity are more anhedonic than those that do not. Such loss of pleasure is a key symptom of clinical depression, and suggests that withdrawn horses are indeed in a depression-like state.

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

All these findings still do not demonstrate with certainty that horses in human care can become clinically depressed: the quality and quantity of current evidence are not yet sufficient to conclude this. However, these data are sufficiently consistent with this hypothesis to make additional research very worthwhile, especially into the breadth and depth of anhedonia in affected horses, the existence of other key symptoms listed by the DSM, and the factors that trigger or reverse the appearance of withdrawn states. Given the range of species and number of individuals it has been suggested may be affected – not just horses but also captive primates and perhaps even pet dogs and cats – the welfare implications of animal depression provide a clear rationale for conducting such research. To end we therefore discuss how future research into animal depression might progress, starting with the assessment of pleasure and its loss.

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

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

The third crucial avenue of research is to investigate predictive validity: the aetiology and response to therapeutic agents of depression-like conditions in horses and other animals. Since aversive early life experiences and stressful life events act as risk factors in humans (*e.g.* Gilmer and McKinney, 2003; Tafet and Bernardini, 2003; Siegrist, 2008), they might therefore do so in animals (see e.g. Camus et al., 2013b). In working horses, variables to investigate should include weaning age/practices (e.g. Visser et al., 2008); time spent in isolation (e.g. McGreevy et al., 1995; Cooper et al., 2000); painful conditions like vertebral problems (e.g. McGreevy and McLean, 2005; Hausberger et al., 2009; Lesimple et al., 2010); and exposure to stressful working conditions (e.g. Hausberger et al., 2009; Lesimple et al., 2010; Hausberger et al., 2011). How depression-like states in animals respond to social support, anti-depressant drugs and reduced stress should also be investigated. Such work would not only fully test the predictive validity of depression-like states in animals (cf. Belzung and Lemoine, 2011), but also have significant welfare implications by revealing how we can we best prevent and treat such states.

5. CONCLUSION

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

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TABLES

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

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

(*) 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 (*e.g.* feels sad, empty, hopeless) or observation made by others (*e.g.* appears tearful). (*Note:* 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.
5 (*)	Psychomotor agitation or retardation nearly every day (observable by others: not merely subjective feelings of restlessness or being slowed down). 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 p*sychomotor 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.

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

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

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

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).

FIGURE CAPTIONS

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

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

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

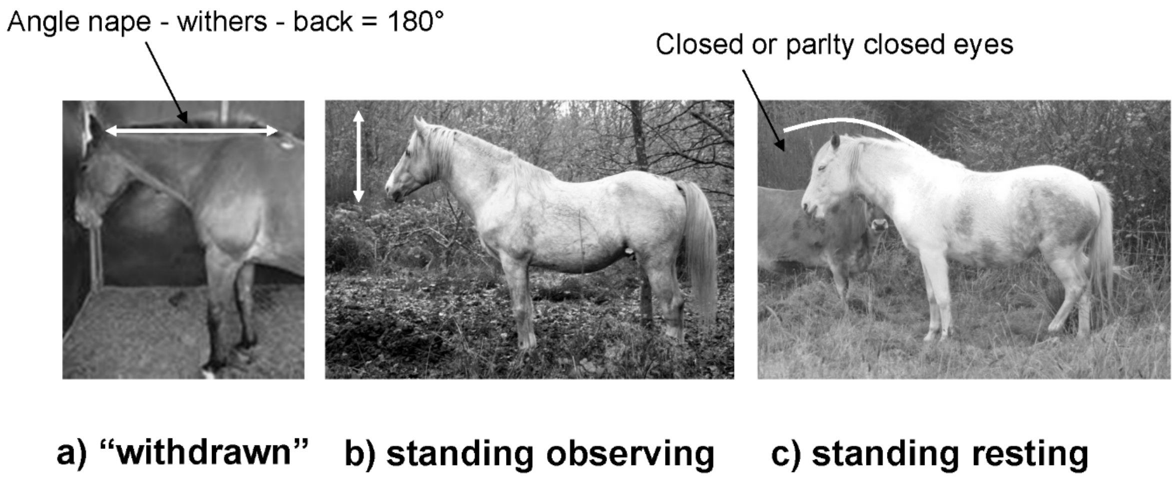
Angle nape - withers - back = 180°
Closed or parlty closed eyes
a) “withdrawn”
b) standing observing
c) standing resting

Fig1

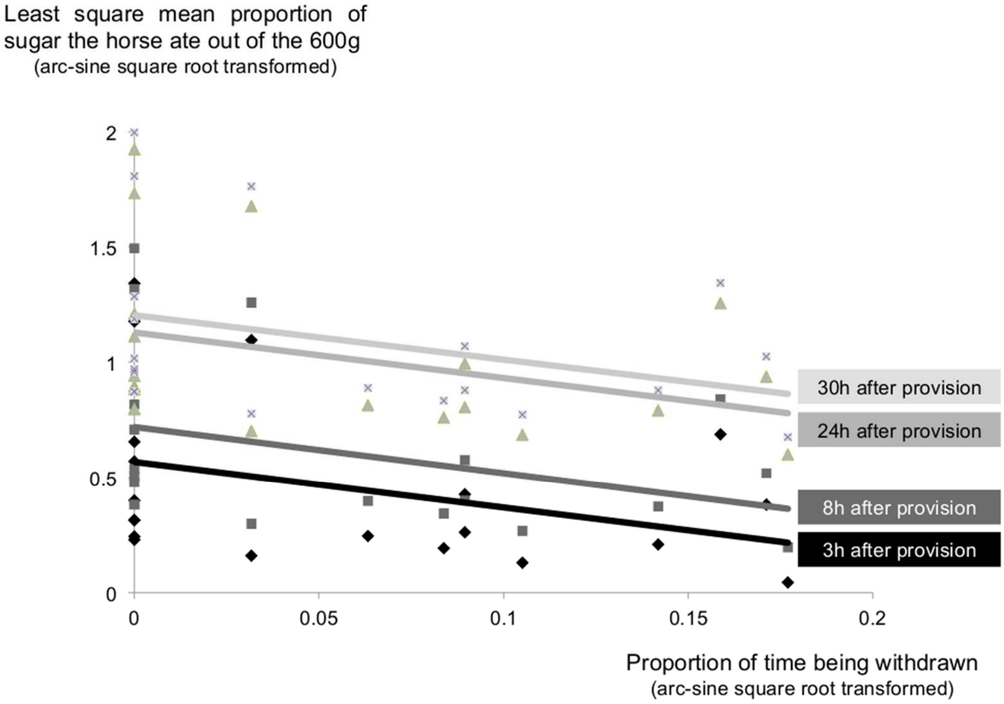
Least square mean proportion of sugar the horse ate out of the 600g
(arc-sine square root transformed)
2
1.5
1
0.5
0
0
0.05
0.1
0.15
0.2
30h after provision
24h after provision
8h after provision
3h after provision
Proportion of time being withdrawn
(arc-sine square root transformed)

Fig2

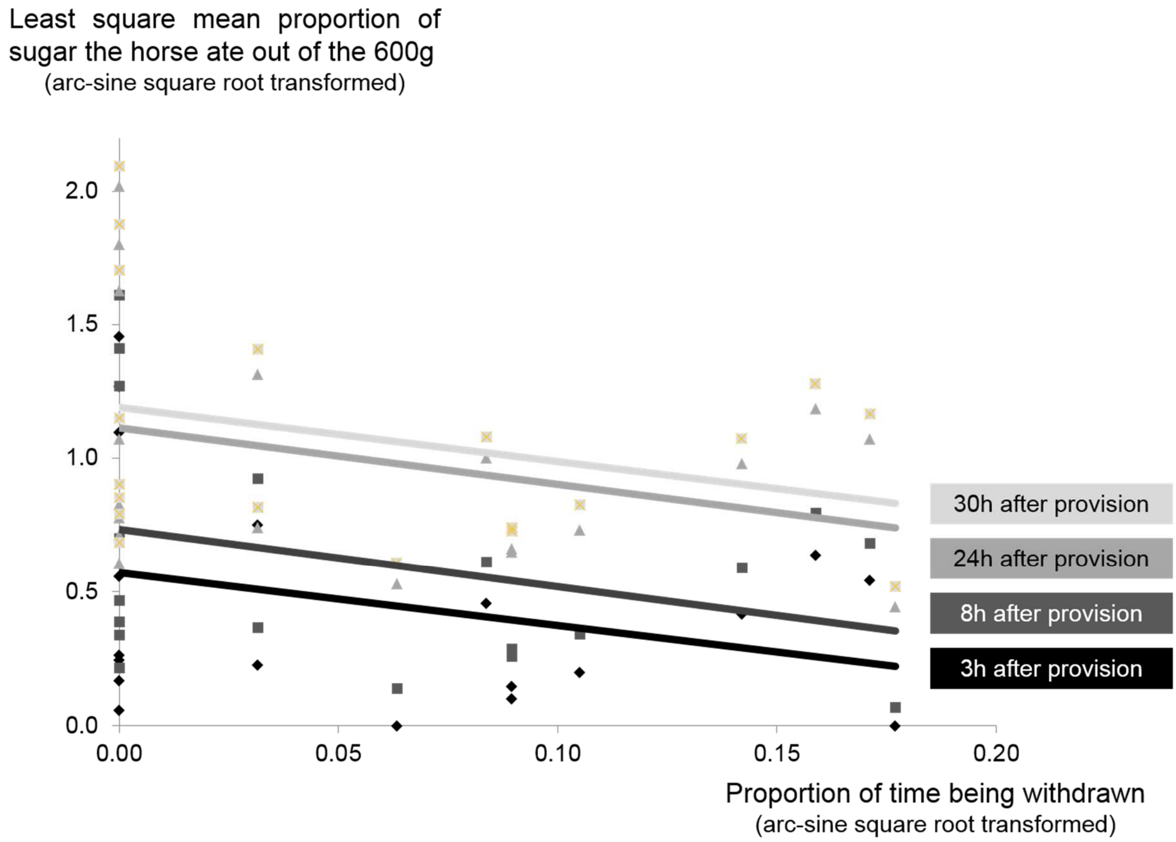
Least square mean proportion of sugar the horse ate out of the 600g
(arc-sine square root transformed)
2.0
1.5
1.0
0.5
0.0
0.00
0.05
0.10
0.15
0.20
Proportion of time being withdrawn
(arc-sine square root transformed)
30h after provision
24h after provision
8h after provision
3h after provision

Fig3