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Effect of a synthetic equine maternal pheromone during a controlled fear-eliciting situation

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Abstract

Horses are known to show fear reactions when confronted with novelty and this can be a considerable hindrance in the context of working situations such as riding, dressage or racing. The aim of the present study was to measure the potential effects of a synthetic analogue of the Equine Appeasing Pheromone on saddled horses when subjected to a stressful situation using a double-blinded, placebo controlled study design. A group of 40 horses was analyzed during this study and horses were divided by sex, breed and reactivity into two homogenized groups. The test, which consisted of walking the horse through a fringed curtain, was selected from a range of tests which are used to assess behaviour for the selection of French breeding stock. Horses that could have been subjected to the test on a previous occasion, and therefore be familiar with it, were not included. Behavioural and physiological parameters were both taken into account with measures of time to go through the curtain, fear related typical behavioural patterns, based on available literature detailed in the bibliography, and heart rate being recorded. Parameters were analyzed by means of Mann–Whitney *U*-test. Significant differences were noticed between the two groups concerning heart rate data during the test ($U_{\mathrm{MeanHR}} = 100.5$, $p_{\mathrm{MeanHR}} = 0.02$; $U_{\mathrm{MaxHR}} = 75$, $p_{\mathrm{MaxHR}} = 0.001$) and during the whole measured period ($U_{\mathrm{MeanHR}} = 67$, $p_{\mathrm{MeanHR}} = 0.005$; $U_{\mathrm{MaxHR}} = 58$, $p_{\mathrm{MaxHR}} = 0.002$). Observation of the animals also revealed less behavioural items characteristic of fear within the treated group. As a result, horses performed the test with a better time performance when they received the pheromone analogue ($U = 62$, $p = 0.002$). The main parameter, area under the HR graph, is based on heart rate measure and performance. Differences

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noticed ($U = 74$, $p = 0.002$) for this parameter lead to the conclusion that horses who received EAP underwent less stress related consequences in terms of their cardiac physiology. As horses are subjected to a number of foreseeable stressful events this study suggests that the use of Equine Appeasing Pheromone could be a significant factor in improving the welfare of this species.

Keywords: Horses; Behaviour; Fear; Pheromone; Heart rate; Performance

1. Introduction

Domesticated horses live in an environment which is far removed from their natural habitat and as result is often lacking in terms of meeting specific equine needs. One potential consequence of this is an increase in levels of anxiety and in related behavioural disorders. When confronted by predators, horses have an instinct to flee and this instinct is heightened in stressful or constraining situations. However, in the modern domestic environment this behaviour is neither adaptive nor desirable for riders. These behavioural inconsistencies between horse and rider could explain various aspects of behavioural problems commonly reported in horses, which are predominantly influenced by fear and often result in detrimental consequences for both the horse and its rider (Broom and Johnson, 1993; Cooper and Mason, 1998; Mc Greevy, 2004).

Neuroleptics have been reported to be commonly used in the treatment of certain behavioural problems in horses (Dodman, 1987, 1988; Mc Donnell, 1998). However, their well identified side-effects (cognitive impairment, muscular tone deregulation, delayed reflexes) as well as the limitations due to anti-doping regulations, make their usage hazardous and maybe even impossible in practice. In addition these molecules only exert an action on the behavioural consequences of the underlying pathology and do not treat the pathology itself.

Behavioural modification programmes (Cooper, 1998; Mills, 1998) are often efficient but they require owner compliance and a minimum level of owner knowledge in terms of learning theories. Evidence relating to the role of misapplied learning theory in the development of equine behaviour problems would suggest that this latter criterion is not commonly met (Mc Greevy, 2001).

Pheromonotherapy (Pageat, 2001; Pageat and Gaultier, 2003) enables a simplification of treatment for anxiety and phobia related issues in various species (dogs, cats, rabbits, pigs) (Gaultier et al., 2005; Griffith et al., 2000; Mc Glone et al., 1993) but its effectiveness in horses has only been studied in isolated cases.

The aim of the present study was to investigate the effects of a synthetic Equine Appeasing Pheromone (EAP) through a double-blinded placebo controlled trial in which a group of 40 horses was submitted to a handling related, potentially fear-eliciting situation (passing through a fringed curtain to enter the stable).

2. Material and methods

2.1. Materials

2.1.1. Subjects

A group of 40 horses, ranging in age from 3 to 30 years, and representing a number of breeds (33 Lusitanian horses, 2 French saddle-bred, 2 Merens ponies, 1 Appaloosa, 2 cross-breed horses) was enrolled on the study. The group was composed of 28 stallions, 4 geldings and 8 mares.

The study was carried out in a ranch located in the south of France where horses are used for both breeding and dressage. All of the horses were housed under similar conditions (individual stalls) on the same breeding farm.

The study was conducted between February and April 2002. Weather conditions were consistent, windy and cold, during the length of the study. Horses that could have been previously subjected to a similar test were not included in this trial.

2.1.2. Group composition

The effects of EAP were evaluated in a double-blinded placebo controlled study. Subjects were divided by sex, breed and ‘‘reactivity’’ and then randomly assigned to the two treatment groups. ‘‘Reactivity’’ was estimated by the trainer, who is a well respected dressage rider, taking into account the intensity of the response of the individual horses when placed in a fearful situation. This parameter was composed of three categories: ‘‘calm’’ (absence of reaction)/‘‘signs of fear’’ (adapted reactions)/‘‘shy’’ (excessive reactions). Distribution of ages in the two groups is presented in Table 1.

3. Material and methods

3.1. Procedure

3.1.1. Treatment

EAP was provided using a spray bottle with a mechanical spraying device. It contained EAP at 0.1% in aqueous solution with preservatives (pH 6.7). The same kind of spray bottle with only aqueous excipient was used for the placebo group (pH 7). The two treatments were transparent and odourless for humans.

Table 1
Distribution of animal ages (years) in the two groups

Parameters	Treatment groups	
	Placebo group ($n = 20$)	EAP group ($n = 20$)
Mean	10.80	13.05
Median	9.5	12
Extreme	3–30	6–23

The treatment, which consisted of two sprays per nostril, was administered to each horse in its stall, 18 min before the beginning of the test. During the period between treatment and commencement of the test (18 min) no social interaction with humans was permitted.

3.1.2. Behavioural test

The fear-eliciting test used in this study was used to collect information relating to response to handling and reaction to a novel object (Bouissou, 1999; Le Scolan et al., 1997; Wolff and Hausberger, 1997). It consisted of placing a fringed curtain outside the stable building and then walking the horses through this curtain in order to enter the stable. The test is based on one of the tests used by the French institution, the Haras Nationaux, for rating horses' behaviour during the breeders' selection process.

The test was divided into five stages:

1. The horse was led to a neutral point of the stable where heart rate monitoring equipment (Polar Horse Trainer Advanced™) was attached.
2. The horse then remained in this area for 2 min (Phase R1).
3. The horse was then led outside, to a place located nearly 20 m away from the curtain (moving phase).
4. The horse remained in this place for a 2 min rest (Phase R2).
5. The horse was led through the curtain by the owner (event phase). After 150 s of unsuccessful proceeding through the course, an assistant intervened with a long whip. After 240 s, the curtain was partially removed. After 300 s, the test was stopped and the trial deemed as a failure.

3.1.3. Parameters

3.1.3.1. Behavioural parameters. Behavioural parameters consisted of two performance measures (Le Scolan et al., 1997; Visser et al., 2003). The first recorded parameter was the time needed for entry (TE). The second parameter was referred to as time with penalty adjustment (TPA) and this was directly derived from the previous measure and was calculated by making penalty adjustments which related to the need for interference in the trial. A 30 s penalty was added if the use of the long whip was required to enter the stall. A second penalty of 30 s was added when the curtain was partially removed and finally a third penalty of 90 s was added when the animal failed the test.

The entire trial was videotaped. The behavioural patterns characteristic of fear were quantified (Table 2) (Bouissou, 1999; Crowell-Davis, 1986; Kiley-Worthington, 1987; Mc Greevy, 2004; Mills and Nankervis, 1999; Rowan, 1988).

In order to emphasise the fear reactions recorded on the video tapes the horses were divided into four categories relating to their entry times: fast (horses with performances

Table 2
Behavioural items categories

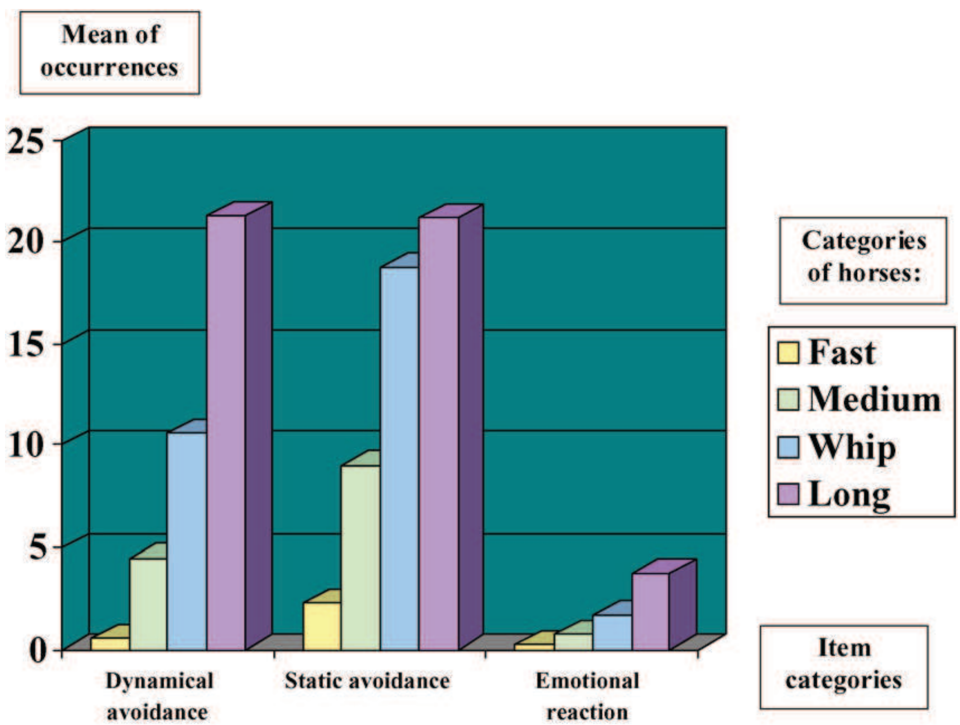
(1)	Dynamic avoidance: Canter, go back, run out, walking sideways
(2)	Static avoidance: Stop or stamping, body weight on the hind-quarters, tail adducted on the rump
(3)	Emotional reaction: Neighing, defecation, involuntary jump, trembling hind-quarters

between 0 and 30 s), medium (between 31 and 150 s), whip (horses that entered the stall encouraged by the presence of the whip, i.e. between 150 and 240 s) and long (between 240 and the end of the trial, i.e. 300 s).

Means of occurrences were calculated for each item category and for each category of horses (Table 3).

3.1.3.2. Physiological parameters. Physiological measurements (Cann et al., 1988a,b; Jezierski et al., 1999; Moberg, 1987) included data describing the heart rate during the event phase and the totality of the trial: mean heart rate (MHR), maximum heart rate (MaHR), minimum heart rate (miHR) and standard deviation of heart rate (HRV). The heart rate was recorded with a Polar Vantage ND system (Polar Electro OY, Kemple, Finland). This heart rate recording system is made of an electrodes belt with a built-in

Table 3
Behavioural observations: mean of occurrences by category of horses



Categories of horses		Fast	Medium	Whip	Long
Number of horses		4	7	17	6
Means of occurrences	Dynamical avoidance	0.5	4.43	10.59	21.33
	Static avoidance	2.25	9	18.76	1.70
	Emotional reaction	0.25	0.71	21.17	3.67

transmitter and a wristwatch receiver. This watch recorded data that was then downloaded via a Polar Interface (Polar Electro OY) to a PC after each group of five horses.

3.1.3.3. Main parameter. The main parameter assessing the efficacy of the product offers the advantage of being based on both performance (TE) and physiological measure (MHR during ‘‘event phase’’). It corresponds to the area under the HR graph. This area was considered to provide a good evaluation of stress related consequences in terms of the horses' cardiac physiology.

All other parameters (performances and physiological measures) are considered as secondary parameters.

3.1.3.4. Statistical analysis. Physiological parameters and the main parameter were tested using a Mann–Whitney *U*-test with a *p*-value less than 5%.

4. Results

4.1. Demographic data

It was necessary to compare the means of the age in the two groups and therefore two verifications have been conducted: the normality of the data distribution and the homogeneity of the variances. Variances were found to be homogenous (Fisher, DL = 19, $F = 1.41$, p NS). Moreover, as data distribution was not normal a Mann–Whitney *U*-test was used ($U = 143.5$; p NS) which permitted the conclusion that there was no significant difference, with $\alpha = 5\%$, between the two groups (Table 1).

4.2. Effects of the fear-eliciting test on studied parameters

Passage through the curtain elicited the display of fear related parameters in horses from each of the two groups (Table 3), and an elevation of heart rate during event phase (Table 4). This elevation cannot be attributed to physical exercise since the test only involved a 20 m walk. Horses with the worst time performances also showed more signs of avoidance and more emotional reactions (in frequency and in types of behavioural patterns) (Table 3).

4.3. Effects of EAP on behavioural parameters

Results showed a clear effect of the EAP treatment on the performance: horses in the EAP treated group needed less time to enter the stall than horses in the placebo group ($U = 62, p = 0.002$) (Table 5). After penalty adjustment, data showed that horses in the EAP group were easier to manage ($U = 61$, $p = 0.002$).

When the horses walked through the curtain there was a positive correlation between the time needed and the display of fear-related behaviours (Table 3). However, no statistical analysis was feasible on this data because all of the parameters were time dependant, i.e. linked. Therefore, these results should be qualified as ‘‘observations’’.

Table 4
Heart rate results

Parameter	Means +/- Standard deviation		U	P-value
HR Event phase	Placebo Group (N)	EAP Group (N)		
MHR (Bpm)	112.2+/-24.5 (19)	95.2+/-19.9 (17)	100.5	0.02
MaHR (Bpm)	172.1+/-24.6 (20)	136.7+/-31.1 (17)	75	0.001
miHR (Bpm)	61.5+/-22 (19)	48.9+/-12.2 (17)	119.5	Ns
HRV (Bpm)	28.7+/-7.3 (19)	24.7+/-10.4 (19)	121.5	Ns

Parameter	Means +/- Standard deviation		U	P-value
HR Entire test	Placebo Group (N)	EAP Group(N)		
MHR (Bpm)	87.2+/-21.9 (18)	68.6+/-14.5 (17)	67	0.005
MaHR (Bpm)	175.4+/-23.2 (18)	144.7+/-23.5 (17)	58	0.002
miHR (Bpm)	43.7+/-11.6 (18)	55.3+/-69.7 (17)	119	Ns
HRV (Bpm)	34.5+/-8 (18)	29.5+/-8.7 (17)	106	Ns

4.4. *Effects of EAP on heart rate parameters*

Although water and ethanol (50/50, v/v) were used systematically to increase contact between the electrodes and the skin of horses, some heart rate data were missing for five horses: data were missing for one mare in the EAP group and were partially missing for one mare and one stallion in each group.

Table 5
Performances results

Parameter	Means +/- Standard deviation		U	P-value
Performances	Placebo Group (N)	EAP Group (N)		
TE (Time for Entry)	203.3+/-66.4 (20)	114.7+/-75.6 (20)	62	0.0002
TPA (Time with Penalty Adjustment)	253.25 (20)	131.2 (20)	61	0.0002

Table 6
HR graph results

Parameter	Means +/- Standard deviation		U	P-value
HR Graph	Placebo Group (N)	EAP Group (N)		
Area	24015+/-13256.1 (19)	11785.8+/-8400.8 (19)	74	0.002

MHR and MaHR were significantly greater for horses in the placebo group during the event phase ($U_{MHR} = 100.5$, $p_{MHR} = 0.02$; $U_{MaHR} = 75$, $p_{MaHR} = 0.001$), but miHR and HRV did not show any difference. For the entire trial, MHR and MaHR showed a significant difference between the two treatment groups ($U_{MHR} = 67$, $p_{MHR} = 0.005$; $U_{MaHR} = 58$, $p_{MaHR} = 0.002$) (Table 4).

4.5. Efficacy assessment (main parameter)

Results coming from the analysis of the HR graph differed significantly between the two groups ($U_{HR} = 74$, $p_{MHR} = 0.002$). Reactions to the test were smaller in the EAP group than in placebo group (Table 6).

5. Discussion

The handling test used in this study, in accordance with the bibliography (Le Scolan et al., 1997; Rowan, 1988; Vierin et al., 1998; Visser et al., 2001, 2003), produces a scenario in which fear related behavioural responses can be studied. Compared to open-field tests a handling situation is known to reveal fearfulness (emotional reactions) of individuals and not gregariousness. All the chosen parameters indicated that the fringed curtain generated stress related indicators which were both behavioural and physiological. This homogeneity was also identified through the decreasing level of stress related indicators identified in those horses which received the pheromonal treatment. Horses in the EAP treated group stopped less frequently when confronted by the fringed curtain and hesitated for a shorter time. As a consequence, they had better performances. Furthermore, measurements of heart rate showed that these horses had lower MHR and MaHR during the event phase but also during the totality of the trial. Results of the HR graph illustrate the consequences of stress, which are evaluated here in terms of the biology of the individuals: this test demonstrates that there are far fewer consequences in relation to the nervous system of horses treated with the pheromonal analogue. Naturally occurring EAP is perceived by the offspring of a lactating mare but the results of this trial suggest that horses are still sensitive to EAP in adulthood.

The loss of heart rate data as a result of the practical recording difficulties (related to the loss of connection between the horses' skin and electrodes) has resulted in a blurring of the differences between the two groups in the statistical analysis. The consequences of these difficulties were particularly unfavourable in respect of the pheromone treated group and therefore do not alter the conclusions in any way that could be seen to bias the results in favour of the pheromone.

It should be noted that the population of horses used in this study consisted mostly of Lusitanian bred stallions. However, seven horses that did not belong to this breed were equally spread out between the categories of performance. No significant difference (with a p-value less than 5%) was found concerning the effect of sex on performances. Stallions are thought to be more difficult to handle and we can assume from the efficacy of pheromonotherapy on stallions that a similar efficacy can be expected on mares and geldings.

The use of EAP seems to be appropriate in a stressful and specific situation as a mean of limiting behavioural and autonomic manifestations of fear and stress which often prevent riders, blacksmiths or veterinarians from managing the horse in a safe and satisfying manner.

Results of the physiological parameters appear to be consistent with the observations made in various species treated with pheromones belonging to the appeasines range (Gaultier et al., 2005). Indeed, we noticed a stabilization of heart rates, and limitation of the extent of cardiac emotional reaction.

Two extra measures could improve the quality of the results in this trial. Firstly, keeping the heart rate recording system on the horses in order to take continuing measurements after the test in a quiet environment would have provided a means of measuring the speed of recovery. Secondly, the great variability recorded in heart rates on resting horses would justify the recording of reference heart rate values for the horses used in this test and such measurements would have enabled the use of each animal as its own reference and to thereby increase the value of the statistical analysis. However, the significance of the differences between the two groups was sufficient to reduce the need for such additional data.

The results of this study suggest that EAP is a useful tool to assist in the completion of specific tasks which need to be carried out in a potentially stressful context. Its application in situations such as shoeing, introduction into a new environment, non-painful veterinary manipulations and introduction to new exercises should therefore be considered. However, taking into account the time lag of around 20 min between applications of the product and observing its effects it is suggested that the use of the EAP will be of optimum benefit in foreseeable stressful events.

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