Pharmacology, Biochemistry and Behavior 94 (2010) 329–332

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effect of perphenazine enanthate on open-field test behaviour and stress-induced hyperthermia in domestic sheep

Cecilia Pedernera-Romano [a,*], José L. Ruiz de la Torre [a], Llorenç Badiella [b], Xavier Manteca [a]

[a] Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Spain
[b] Servei d´Estadística, Universitat Autònoma de Barcelona, Spain

ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form 14 September 2009
Accepted 21 September 2009
Available online 30 September 2009

Keywords:
Fear
Open-field test
Stress-induced hyperthermia
Perphenazine enanthate
Neuroleptic
Behaviour
Sheep

ABSTRACT

The open-field test (OFT) and stress-induced hyperthermia (SIH) have been used to measure individual differences in fear. The present study has been designed as a pharmacological validation of OFT and SIH as indicators of fear in sheep using perphenazine enanthate (PPZ), a long-acting neuroleptic. Twenty four ewes of two breeds, Lacaune and Ripollesa, were tested in an arena measuring 5 m × 2.5 m. Treatment group received one dose of 1.5 mg/kg of PPZ and control group received sterile sesame oil. All animals were tested for 10 min and behaviours were recorded. Rectal temperature was measured at the beginning (*T*1) and at the end (*T*2) of the test. SIH was defined as the difference between *T*2 and *T*1. Sheep were tested on days 1, 2, 3, 4, 7 and 9 after PPZ injection. Variables were analysed using a mixed model. PPZ decreased bleats on days 2, 3, 4 and the SIH response on days 2 and 3. Breed differences were observed. Treated animals showed positive correlations between SIH and bleats; squares entered; attempts to escape and negative correlation between SIH and visits to the food bucket. Our results suggest that behaviour and SIH on the OFT are useful measures of fear in sheep.

1. Introduction

There is a large variety of psychological, physiological and chemical inputs requiring specific responses in a threatening situation. We have used a combination of tests to measure behavioural and physiological changes due to a fear situation on sheep. Open-field test (OFT) is the most commonly used test to measure fearfulness in animals and was developed for laboratory animals. The open-field test or arena test generally consists in separating an individual from its co specifics and introducing it into an unfamiliar and poor environment (Gray, 1987). In sheep a high number of bleats, and of rearings/jumpings against the wall in the OFT have been interpreted as "distress responses" induced by isolation (Le Neindre et al., 1996; Romeyer and Bouissou, 1992; Vandenheede et al., 1998). Exploratory behaviours directed to an object reflect a low level of fear combined with a high motivation to explore (Boissy and Bouissou, 1995; Moberg and Wood, 1982; Romeyer and Bouissou, 1992; Vandenheede et al., 1998). In general, higher levels of locomotor and vocal activity have been taken to indicate higher levels of fear in sheep exposed to the same challenging situation (Erhard et al., 2004; Romeyer and Bouissou, 1992; Vandenheede et al., 1998; Vierin and Bouissou, 2003).

Stress-induced hyperthermia (SIH) is mediated by the autonomic nervous system and is well known to occur prior to and during exposure to stress- and/or anxiety-inducing situations (Spooren et al., 2002). In rats the SIH paradigm is sensitive to classic and potential novel anxiolytics, while being unable to detect anxiogenic effects and effects of antidepressants (Bouwknecht et al., 2007).

Perphenazine enanthate (PPZ) has been successfully used in several species as a tranquillizer during the period of adaptation to new environments to reduce stress (Ebedes and Raath, 1999). It is considered a long-acting tranquillizer with sedative effects starting from 10 to 16 h after injection and lasting from 7 to 10 days. Their effectiveness varies among different species and among individual animals of the same species and the duration of its effects is dose dependent (Ebedes and Raath, 1999). Long-acting neuroleptics (or tranquillizers) were developed initially for the treatment of psychoses in non-compliant psychiatric patients. Their long action is achieved by esterifying the active compound and dissolving the ester in vegetable oil, allowing delayed hydrolysis and slow absorption into the blood (Lingjaerde, 1973).

Our hypotheses are that isolation produces fear in a gregarious species such as sheep and that the use of a tranquillizer will reduce fear related behaviours and SIH response. The aim of the study was to investigate the influence of a long-acting neuroleptic (LANs) on SIH and behavioural responses in the open-field test (OFT) in order to further validate both measures as indicators of fear in sheep.

2. Materials and methods

2.1. Animals and standard housing conditions

Twenty four ewes of one year and six months of two different breeds: Lacaune ($n = 12$), mean ± sd weight was (68.82 ± 5.6 kg) and

* Corresponding author.
E-mail address: cecipedernera@hotmail.com (C. Pedernera-Romano).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.09.013

Ripollesa ($n=12$), mean ± sd weight was (50.76 ± 2.24 kg) from the experimental farm of the Univeristat Autonoma de Barcelona were used for this study. This procedure was approved by the Institutional Animal Care and Use Committee of the Universitat Autonoma de Barcelona.

2.2. Experimental procedure

Animals were randomly assigned to the control or treatment group $n=12$ (6 Lacaune and 6 Ripollesa). Animals in the treatment group received an intramuscular injection of 1.5 mg/kg of perphenazine (100 mg mL^{-1}, Perphenazine enanthate, Kyron Laboratories, South Africa) and sheep in the control group received an intramuscular injection of 1.5 mg/kg of sterile sesame oil, in a random order in their home pens. Injections were given in the afternoon, 14 to 16 h before the beginning of the test. Animals were tested in the OFT between 08:30 and 13:00.

2.3. Open-field test and SIH

The ewes were tested in an arena measuring 5 m × 2.5 m marked out in a grid of squares each measuring 0.83 m × 0.83 m. Water and a familiar food were placed in the arena in a familiar bucket against the wall facing the entrance door. The sheep to be tested was taken from a waiting pen by a person and allowed to enter the arena, where it remained for a period of 10 min. Behaviour in the arena was recorded by an observer who was not visible to the experimental animals. Animals were individually subjected to the test. The test was run on days 1, 2, 3, 4, 7 and 9 post injection. Sheep were tested once a day always in the same order. Rectal temperature was measured for 90 s when the animal entered the arena ($T1$) and again when the OFT was finished ($T2$). SIH was defined as $T2$ minus $T1$.

2.4. Measurement of behaviour

The following behaviours were recorded: number of bleats, number of squares entered, visits to the food bucket, visits to the water bucket, number of times the ewe sniffed the ground or the walls (exploring), urinations, defecations, attempts to escape and events of grooming.

2.5. Statistical analysis

Statistical analysis was performed using SAS® v9.1.3 (SAS Institute, Cary, NC, USA) and significance level was set at 0.05. Response variables were summarised using mean ± standard deviation and sample size when necessary. Spearman correlations between response variables (averaging measures from the same animal) were obtained as a descriptive measure of association. Continuous response variables ($T2$, $T1$ and ΔT) were analysed using a mixed model. The variable animal was considered as a random factor in order to take into account the repeated measures on the same animal. Breed, treatment and day were included as fixed effects (categorical), as well as all possible interactions. This model allowed us to examine all main effects and possible interactions, and to compare different levels as well as combination of levels for different explanatory variables (breed, treatment and day). Due to its balanced nature, the design does not lead to confusing interpretations when non-significant interactions remain in the model. Finally, graphical plots of the mean evolution of each parameter for each treatment group and breed were obtained using least square means and their standard error as obtained from the model.

Response variables based on counts which showed sufficient variability (bleats, visits to the food bucket, squares entered and exploring) were analysed using the previous mixed model, assuming an appropriate distribution for the response variables. Generalized linear mixed models were used assuming Poisson distribution or negative-binomial distribution depending on the presence of overdispersion. Graphical plots were also obtained using the same summary indexes. The effect of breed and treatment on response variables with low degree of variability (visits to the water bucket, urinations, defecations, attempts to escape and grooming behaviour) was analysed averaging measures from the same animal and using Kruskal–Wallis non-parametric test. Because of the exploratory character of the present study, multiple comparison corrections were not applied.

3. Results

Animals remained in general good health throughout the duration of the experiment and adverse effects were not seen. Although some minor alterations in behaviour were noticeable, a tranquillizing effect, which has been shown in wild animals, was visible only on the first day after injection in some of the ewes that showed a "dizzy walk".

None of the measures showed a simple treatment effect but, there were interactions with breed or day effects. Table 1 shows the statistically significant effects on each variable. Interactions must be interpreted carefully, since they represent the effect of the combination of several factors. For example, interaction between day and treatment would mean a different pattern of evolution between treated and control animals.

3.1. PPZ and behaviour

On the first two days after injection no differences were observed between treatment and control groups for any of the breeds. Afterwards, the pattern of behaviour was different between breeds and some differences between control and treatment groups appeared. Treated animals bleat lees than control (Fig. 1), and in Lacaune sheep this effect was statistically significant on days two, three and four, whereas in Ripollesa ewes no statistically significant differences in this behaviour were found between control and treatment groups.

Squares entered or locomotion; visits to the food bucket and exploratory behaviour were affected by the repetition of the test, day effect, and both breed behave differently (Table 1). Locomotion tended to decrease with the repetitions of the test. Exploratory behaviour was low on the first four days but it increased on days 7 and 9. No treatment effect was seen in these variables.

Table 1
Effects of breed, day and treatment on behaviour and body temperature.

	Effect	*F* value	*p* value
Bleats	Day	9.13	<0.0001
	Breed	5.94	0.0242
	Breed*day	11.69	<0.0001
	Treatment*breed	5.96	0.0164
Squares entered	Day	8.21	<0.0001
	Breed*day	3.62	0.0047
Food bucket	Breed	13.59	0.0015
	Breed*day	3.08	0.0127
Exploring	Day	3.3	0.0085
	Breed*day	5.63	0.0001
T1	Breed*day	3.81	0.0031
T2	Day	2.38	0.0442
	Breed	11.09	0.0033
	Breed*day	4.16	0.0018
	Treatment*day	2.36	0.0459
ΔT	Day	6.49	<0.0001
	Breed	23.9	<0.0001
	Treatment*day	3.63	0.0048

The term day represents the evolution over the 6 days of the study, breed is a categorical variable (Lacaune and Ripollesa) and treatment includes control and PPZ groups. Significative *F* values are indicated.

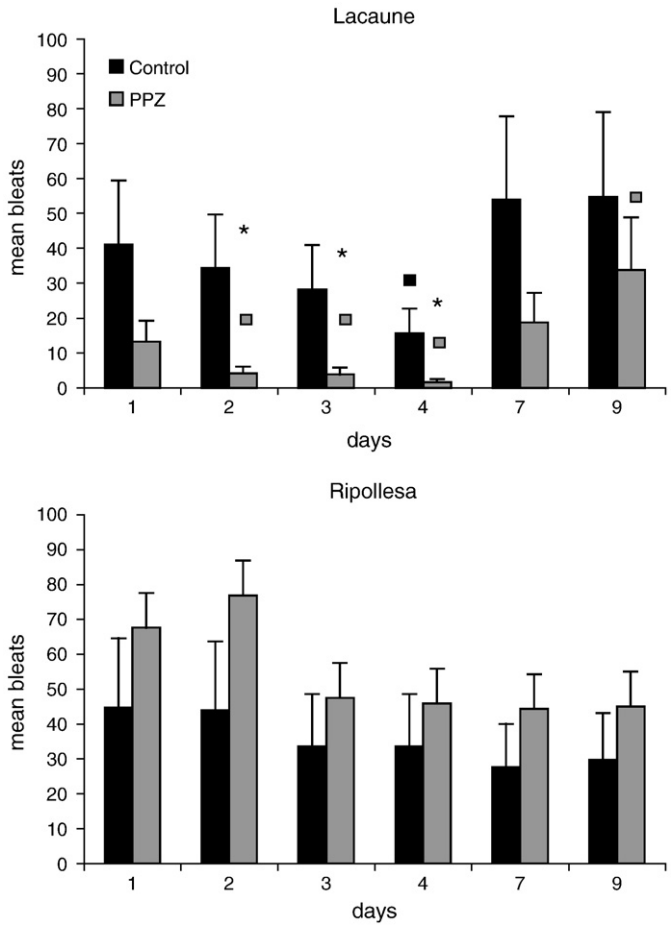

Fig. 1. Mean ± SE of bleats in treatment and control ewes from day 1 to 4 and on days 7 and 9 post PPZ injection. Significance levels are indicated by asterisks (*differences between treatment and control $p<0.05$) and by squares (□ differences from day one on each group control and PPZ $p<0.05$).

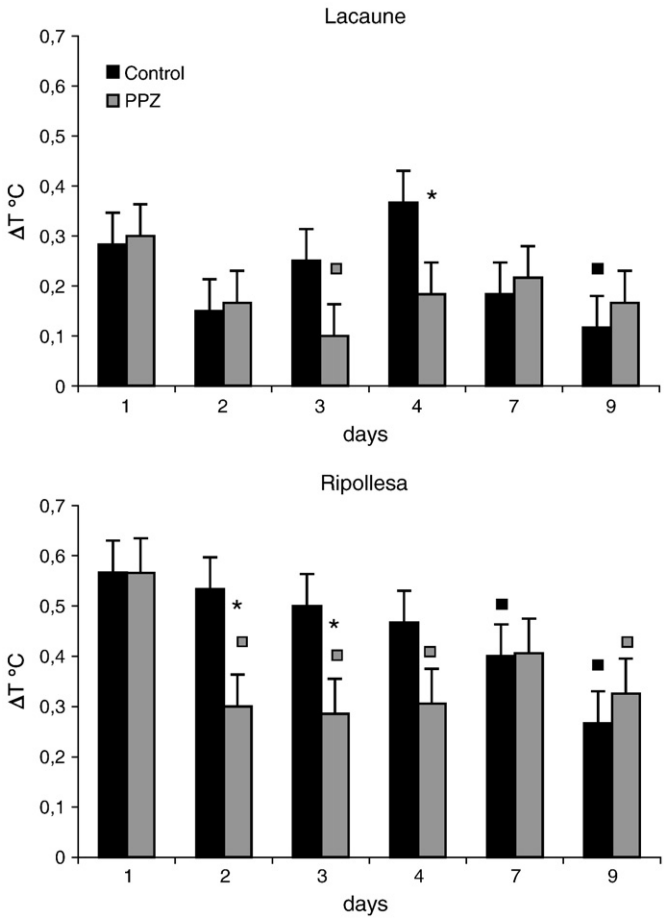

Fig. 2. Figure Mean ± SE of SIH in treated and control ewes from day 1 to 4 and on days 7 and 9 post PPZ injection. Significance levels are indicated by asterisks (*differences between treatment and control $p<0.05$) and by squares (□ differences from day one in control and PPZ group $p<0.05$).

3.2. PPZ and body temperature

Treatment animals showed positive correlations between ΔT and bleats ($r=0.651$, $p=0.033$); squares entered ($r=0.692$, $p=0.012$); attempts to escape ($r=0.666$, $p=0.017$) and negative correlations between SIH and visits to the food bucket ($r=-0.582$, $p=0.046$). No correlation was found in control animals.

Perphenazine enanthate affected the SIH response. Both breed showed a similar pattern. In Lacaune sheep this effect was statistically significant on day four. In Ripollesa ewes the effect was statistically significant on days two and three (Fig. 2). Treated animals (Lacaune 0.188 ± 0.066 and Ripollesa 0.365 ± 0.107) showed a lower SIH than control animals (Lacaune $0.225\ \pm0.0929$ and Ripollesa 0.455 ± 0.108). In Ripollesa sheep, differences in ΔT between treatments were more pronounced than in Lacaune sheep.

4. Discussion

According to our data bleats and SIH were the only two parameters affected by the PPZ treatment. Stressful and painful stimuli produce a series of behavioural and physiological responses in animals, often including intense vocalisation. Consequently, assessment of vocalisation is a common measure in many pharmacological and ethological studies of stress. The vast majority of these studies have been conducted in rodents, in particular in rats (Sanchez, 2003). In our study, Lacaune ewes treated with PPZ emitted fewer bleats than control Lacaune ewes. To the best of our knowledge, the effect of PPZ on vocalisations in sheep had not been described before. In rats, classical antipsychotics like haloperidol have no or a weak inhibitory effect on footshock-induced ultrasonic vocalisation, this effect being shown only at doses that induce catalepsy (Sanchez, 2003) .Other effects of perphenazine reported in sheep (Tomlinson et al., 1982) using a 250-mg intrajugular injection of perphenazine demonstrate that this drug does not facilitate acceptance of alien lambs by ewes that have access to their own natural lambs. However, tranquillization can be used to facilitate fostering of alien lambs if natural lambs are absent because it reduces the aggressiveness of the ewe.

The OFT allows the evaluation of the effects of drugs on different aspects of the behaviour of animals and their underlying motivation. The number of OFTs that an individual animal undergoes greatly influences its behaviour and, likely, its underlying motivation. (Choleris et al., 2001). Day effect on behaviour represents the influence of repeating the test, it can be considered that after numerous runs through the test, sheep may not perceive it as a novel environment so become less fearful and for example more exploratory. As LANs have a direct depressive effect on the hypothalamus and can reduce locomotor activity, it could be argued that animals are not less stressed but that it is just their physiological response to stress which is in some way altered (Diverio et al., 1996). In our work, treatment reduced attempts to escape but this was not due to the effect on locomotor activity, that was not affected, but to a different perception of the stressful situation. One reason for the lack of predicted effects on activity and the rest of behavioural variables, except bleats, may be dose of drug used. However, the doses we used are those conventionally used (Ebedes, 1993), and produced tranquillization without the extrapyramidal symptoms associated with overdosing.

Large differences in fear responses of ruminants have been observed between breeds (review Boissy et al., 2005). The origins of these differences are unclear, but may have arisen from direct selection for behavioural characteristics or as correlated changes in response to selection for productivity (Wolf et al., 2008). Differences between breeds in reactivity towards potentially fear-eliciting situations have often been reported in adult sheep. In general less domesticated "light" breeds are reported to be more reactive than commercial "heavy" breeds (Hansen et al., 2001; Romeyer and Bouissou, 1992). This may explain the differences between breeds in their behavioural response to treatment, Ripollesa ewes probably requiring a higher dose of PPZ.

From our results we can say that treatment with perphenazine enanthate may have an effect on reducing hyperthermia produced by the stress situation. As a preliminary study we measured rectal temperature on ewes in their home pens, measuring temperature for 1.5 min $T1$ and then waiting 10 min and measuring $T2$. ΔT showed no differences so we can infer that the differences in response obtained in the test are due to isolation and novelty and not only because of the insertion of the rectal probe (Bouwknecht et al., 2007). The phenothiazines, of which perphenazine is a member, are widely reported to affect thermoregulation (Mann and Boger, 1978; Thorne, 1980), and we observed some effects in both breeds in SIH. Fick et al. (2006, 2007) didn't find an effect of PPZ on body temperature of blue wildebeest nor of goats (Fick et al., 2007). We found SIH differences two, three and four days after injection. Body temperature of rats exposed to challenges to noxious heat and pressure and injected with perphenazine decreased by 0.5 °C for up to 17 h after injection (Fick et al., 2005). Effect of LANs, on thermoregulation may vary depending on the stimuli and may last longer in different species. Using zuclopenthixol acetate, Read et al. (2000) measured rectal temperature after handling wapiti for blood sampling, and concluded that tranquillized individuals were less stressed by the handling and so exhibited a smaller increase in body temperature than did untranquillized animals. Further research is needed.

All antipsychotic drugs (also termed neuroleptic drugs or major tranquillizers) are dopamine antagonists (Pleuvry, 2004). In the dopaminergic system, the dopamine D_2 receptor has been extensively studied. Stimulation (Groenink et al., 2003) and blockade of the dopamine D_2 receptor (Borsini et al., 1989; Lecci et al., 1990; Zethof et al., 1995) as well as inhibition of DA reuptake into the cells (Zethof et al., 1995) did not alter the stress response (for a review see Bouwknecht et al., 2007). But ΔT was lower on some days of treatment that can suggest an effect of PPZ on the SIH response. The positive correlation of ΔT with bleats, locomotion and attempts to escape suggest this difference may be due to the stress produced by the test. Further research will be needed to evaluate the effects of PPZ on SIH.

The effect of the PPZ was expected to last for 7–10 days (Ebedes and Raath, 1999) owing to the depot release of the drug profile but in general from day 7 the responses we measured seem not to be affected by the drug. At the same time the OFT situation may not be considered as fearful for the sheep anymore because of a possible adaptation throughout repetitions.

In conclusion, our results indicate that the use of the long-acting neuroleptic perphenazine enanthate may be useful in reducing the effect of stress during and isolation or novelty situation. The use of a tranquillizer allowed us to validate both behaviours displayed at the OFT and SIH as indicators of fear. Since it is essential to avoid or, at least, reduce the animals' stress during their management, the use of LANs may become very useful for wild animal handling i.e. transport.

Acknowledgements

Thanks are due to the Servei de Granges i Camps Experimentals of the Veterinary School of the UAB, Barcelona, Spain and the Consejo Nacional de Ciencia y Tecnologia de México (CONACYT) — Fundacion Carolina for the funding of the Ph.D. student. The contribution of the author Llorenç Badiella was supported by grant MTM2006-01477 from the Ministry of Education of Spain. We gratefully acknowledge the practical assistance of Natalia Adell, Déborah Temple and Pol Llonch of UAB.

References

Boissy A, Bouissou M. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Appl Anim Behav Sci 1995;46:17–31.
Boissy A, Fisher AD, Bouix J, Hinch GN, Le Neindre P. Genetics of fear in ruminant livestock. Livest Prod Sci 2005;93:23–32.
Borsini F, Lecci A, Volterra G, Meli A. A model to measure anticipatory anxiety in mice? Psychopharmacology (Berl) 1989;98:207–11.
Bouwknecht AJ, Olivier B, Paylor RE. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. Neurosci Biobehav Rev 2007;31:41–59.
Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001;25:235–60.
Diverio S, Goddard PJ, Gordon IJ. Use of long-acting neuroleptics to reduce the stress response to management practices in red deer. Appl Anim Behav Sci 1996;49:83–8.
Ebedes HYM. The use of long-acting tranquillizers in captive wild animals. In: Mckenzie A, editor. The Capture and Care Manual. Pretoria, South Africa: Wildlife Decision Support Services and the South African Veterinary Foundation; 1993. p. 71–99.
Ebedes HYM, Raath JP. Use of tranquilizers in wild herbivores. In: Fowler M, Miller E, editors. Zoo and Wild Animals Medicine, Current Therapy. Philadelphia: W.B. Saunders; 1999. p. 575.
Erhard HW, Boissy A, Rae MT, Rhind SM. Effects of prenatal undernutrition on emotional reactivity and cognitive flexibility in adult sheep. Behav Brain Res 2004;151:25–35.
Fick LG, Fuller A, Mitchell D. Thermoregulatory, motor, behavioural, and nociceptive responses of rats to 3 long-acting neuroleptics. Can J Physiol Pharmacol 2005;83:517–27.
Fick L, Matthee A, Mitchell D, Fuller A. The effect of boma-housing and long-acting tranquillizers on body temperature, physical activity and food intake of blue wildebeest (Connochaetes taurinus). J Therm Biol 2006;31:159–67.
Fick L, Mitchell D, Fuller A. Long-acting neuroleptics used in wildlife management do not impair thermoregulation or physical activity in goats (*Capra hircus*). Comp Biochem Physiol Part A Mol Integr Physiol 2007;147:445–52.
Gray JA. The psychology of fear and stress. Cambridge: Cambridge Univeristy Press; 1987.
Groenink L, Pattij T, De Jongh R, Van der Gugten J, Oosting RS, Dirks A, et al. 5-HT1A receptor knockout mice and mice overexpressing corticotropin-releasing hormone in models of anxiety. Eur J Pharmacol 2003;463:185–97.
Hansen II, Christiansen F, Hansen HS, Braastad B, Bakken M. Variation in behavioural responses of ewes towards predator-related stimuli. Appl Anim Behav Sci 2001;70:227–37.
Le Neindre P, Boivin X, Boissy A. Handling of extensively kept animals. Appl Anim Behav Sci 1996;49:73–81.
Lecci A, Borsini F, Volterra G, Meli A. Pharmacological validation of a novel animal model of anticipatory anxiety in mice. Psychopharmacology (Berl) 1990;101:255–61.
Lingjaerde O. Some pharmacological aspects of depot neuroleptics. Acta Psychiatr Scand Suppl 1973;246:9-14.
Mann SC, Boger WP. Psychotropic drugs, summer heat and humidity, and hyperpyrexia: a danger restated. Am J Psychiatry 1978;135:1097–100.
Moberg GP, Wood VA. Effect of differential rearing on the behavioral and adrenocortical response of lambs to a novel environment. Appl Anim Ethol 1982;8:269–79.
Pleuvry BJ. Antidepressants and antipsychotics. Anaesth Intensive Care Med 2004;5:354–7.
Read M, Caulkett N, McCallister M. Evaluation of zuclopenthixol acetate to decrease handling stress in wapiti. J Wildl Dis 2000;36:450–9.
Romeyer A, Bouissou M. Assessment of fear reactions in domestic sheep, and influence of breed and rearing conditions. Appl Anim Behav Sci 1992;34:93-119.
Sanchez C. Stress-induced vocalisation in adult animals. A valid model of anxiety? Eur J Pharmacol 2003;463:133–43.
Spooren WPJM, Schoeffter P, Gasparini F, Kuhn R, Gentsch C. Pharmacological and endocrinological characterisation of stress-induced hyperthermia in singly housed mice using classical and candidate anxiolytics (LY314582, MPEP and NKP608). Eur J Pharmacol 2002;435:161–70.
Thorne ET. Immobilization of wild ungulates: considerations and drugs. Biotelem Patient Monit 1980;7:178–87.
Tomlinson KA, Price EO, Torell DT. Responses of tranquilized post-partum ewes to alien lambs. Appl Anim Ethol 1982;8:109–17.
Vandenheede M, Bouissou MF, Picard M. Interpretation of behavioural reactions of sheep towards fear-eliciting situations. Appl Anim Behav Sci 1998;58:293–310.
Vierin M, Bouissou MF. Responses of weaned lambs to fear-eliciting situations: origin of individual differences. Dev Psychobiol 2003;42:131–47.
Wolf BT, McBride SD, Lewis RM, Davies MH, Haresign W. Estimates of the genetic parameters and repeatability of behavioural traits of sheep in an arena test. Appl Anim Behav Sci 2008;112:68–80.
Zethof TJJ, Van Der Heyden JAM, Tolboom JTBM, Olivier B. Stress-induced hyperthermia as a putative anxiety model. Eur J Pharmacol 1995;294:125–35.