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Behavioural changes in dogs treated with corticosteroids

Lorella Notari, Oliver Burman, Daniel Mills

Animal Behaviour, Cognition and Welfare Group, School of Life Sciences, Joseph Banks Laboratories, University of Lincoln, Lincoln LN6 7DL, UK

HIGHLIGHTS

- We aimed to identify behavioural changes in dogs treated with corticosteroids.
- Dogs on corticosteroids showed behaviour associated with a negative affective state.
- In a behavioural test, dogs on corticosteroids avoided a mildly aversive stimulus.
- Dog owners should be advised by veterinarians about behavioural risk management.

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ABSTRACT

In human medicine, psychiatric side effects among patients on corticosteroid therapy are widely reported, but this appears to have been largely overlooked in the animal literature despite glucocorticoids being widely used in veterinary medicine. Therefore the aim of the current study was to identify possible psycho-behavioural changes in dogs treated with corticosteroids. Two different methodologies were used. Firstly, dog owners were asked to fill a 12 item questionnaire aimed at further validating the initial results of a previous survey relating to changes seen when their dog was receiving corticosteroid treatment. In a second study, a population of dogs undertook behavioural tests aimed at objectively identifying changes when receiving corticosteroid therapy.

In the first study, a sample of owners whose dogs were receiving treatment for dermatological, orthopaedic or other conditions evaluated their dogs' behaviour on and off therapy, using a seven point scale. The survey was completed by 44 dog owners with dogs receiving treatment with a range of corticosteroid preparations (mainly prednisolone and methylprednisolone) and 54 dog owners with dogs receiving treatment with other drugs, mainly antibiotics and non-steroidal anti-inflammatory drugs. Dogs under corticosteroid treatment were reported to be significantly less playful, more nervous/restless, more fearful/less confident, more aggressive in the presence of food, more prone to barking, more prone to startle, more prone to reacting aggressively when disturbed, and more prone to avoiding people or unusual situations.

In the second study, eleven "treatment" dogs were tested both before and during corticosteroid treatment with either methyl-prednisolone or prednisolone to assess their sensitivity to a potentially aversive sound stimulus. Eleven control dogs were also tested at the same time intervals in the same environment. Dogs were exposed to a brief dog growl while they explored bowls containing food and their behaviour was video recorded. Treatment dogs were found to investigate the area in the vicinity of the bowls for significantly less time and to eat significantly fewer pieces of food when on corticosteroids, compared to control dogs, after hearing the growl. These results provide the first empirical evidence of possible adverse psycho-behavioural side effects in a veterinary clinical setting following the use of corticosteroids, and suggest the need for concomitant behavioural advice when these drugs are used in general veterinary practise to avoid the risks associated with these changes.

1. Introduction

Glucocorticoids are widely used in veterinary practice but are also among the most important mediators of the stress-response [1,2]. This response has physiological, behavioural, cognitive and emotional components, having the potential to inhibit positively motivated responses and to increase anxiety-related behaviours [3–5]. Glucocorticoids mediate changes in cognition, learning and emotional processes through the activation of glucocorticoid receptors in diverse brain areas from the prefrontal cortex through to the hippocampus, basal ganglia and amygdale [3]. Both excesses and deficits in glucocorticoid can lead to impairment of learned and emotional processes and responses [6].

E-mail address: lorellanotari@lorellanotari.it (L. Notari).

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The therapeutic use of glucocorticoid drugs in companion animals is associated with several well recognized physical side effects including gastro-intestinal problems, suppression of adrenal gland function and increased risk of infections [7,8]; possible psychological side effects have not received much attention beyond a preliminary survey reported by the authors [9]. However, in human medicine, several surveys and case reports have shown that important psychiatric side effects can occur in patients on corticosteroid therapy. It has been suggested that the onset of corticosteroid-induced psychiatric disturbances might be linked to pre-existing individual psychological characteristics such as personality, with these reactions reflecting an extreme version of a patient's usual stress reaction [10–12]. Increasing attention is being given to these effects in human patients, receiving long term treatment [11,13,14]. Neurological toxicity due to the drug itself or the synergistic action of drugs administered concurrently have been postulated for the unexpected behavioural and psychiatric effects of these medications when prescribed for physical diseases [5],these effects are probably most often related to the neurochemical cascades linked to the stress response [14–16].

Alongside studies in human medicine, there is abundant evidence of the influence of exogenous pituitary adrenal hormones on animal behaviour but not specifically in a therapeutic context as might occur in companion animals. Studies in laboratory animals have shown that exogenous glucocorticoids can affect cognitive functions such as learning and memory [6,17–19]. An effect on the emotional states of animals has also been hypothesized with, for example, the experimental administration of corticosteroids to rats appearing to influence their subsequent emotional response to unexpected reductions in reward size [20]. This study showed that rats treated with corticosteroids responded to an unexpected downshift in reward magnitude by showing a significantly greater decrease in their consummatory behaviour – interpreted as an expression of their emotional response – compared to a control group subjected to the same procedure. Many of the behavioural effects of corticosteroids would be expected if these chemicals induced a negative cognitive bias, e.g. a greater sensitivity to potentially threatening stimuli in the environment [21].

Investigating the potential negative behavioural side effects of glucocorticoid drugs in companion animals is clearly important in order to make a full risk–benefit analysis concerning their use, and to ensure that appropriate advice can be given to owners and veterinarians when these drugs are prescribed. Therefore the present study aimed to investigate the effects of corticosteroid therapies on dog behaviour: firstly, through a retrospective study using questionnaire responses concerning the behaviour of dogs when on and off corticosteroid therapy; and secondly, through a case–control study of the responses of subjects in a behavioural test aimed at assessing the animal's response to a potentially threatening sound stimulus.

2. Materials and methods

2.1. Questionnaire study

A 12 item questionnaire was completed by dog owners with dogs receiving or having recently received drug treatment, preferably for dermatological or orthopaedic conditions. The questionnaire was informed by the results of a previous survey [9]. Seven of the 12 items were selected on the basis of the results of a previous survey [9], with five further questions ('fillers') relating to other behavioural changes not identified in the previous survey selected among behaviours that frequently cause complaints by dog owners [22,23], but not thought to be influenced by corticosteroids. These were inserted partly as 'fillers' and to aid validation of target effects [24]. The questionnaire was published via the Internet in both English and Italian, with a paper version also distributed to Italian veterinary clinic clients. Questionnaires were back translated by independent mother tongue translators to assess the consistency of the two versions. The items were scored on a seven point scale with two scores for each question posed: one score for the respondent's perception when the animal was receiving pharmacological treatment for the condition and one for when the animal was not receiving pharmacological treatment (Fig. 1).

The introductory part of the questionnaire gathered demographic data relating to both the owner and their dog, information about the drugs being given to the dog at the time of survey (such as type of drug, time of administration and doses) and information about the type of condition/disease for which it was being used. The respondents were asked to mention all drugs taken in the same period for the same or other concomitant conditions. The questionnaire was to be completed on the Internet and advertised through veterinary associations, pet websites and magazines both in Italy and UK. Paper questionnaires were also distributed in veterinary clinics in the north of Italy.

Questions are illustrated in Table 1 and Items 1 (Play behaviour), 5 (Attention seeking), 7 (Obedience), 8 (Guarding behaviour) and 12 (Mounting behaviour) were added as additional fillers.

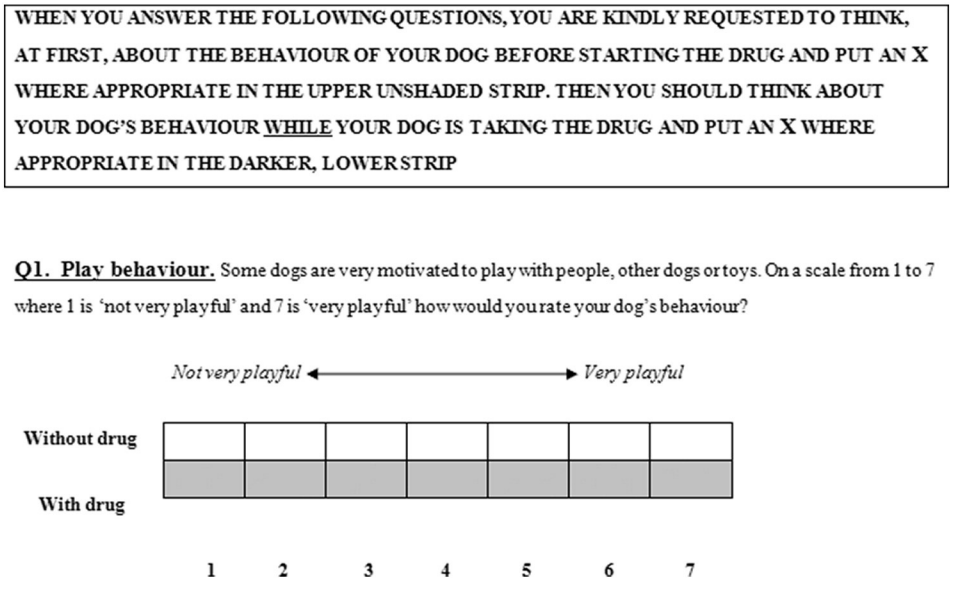

Fig. 1. Example of question and scoring system used in owner questionnaire: Q1 Play Behaviour.

Table 1
Owner questionnaire. Verbatim of questions. Scales 1–7 illustrate the level of expression of the investigated behaviour.

Question numbers	Details of questions
Q1	Some dogs are very motivated to play with people, other dogs or toys. On a scale from 1 to 7 where 1 is 'not very playful' and 7 is 'very playful' how would you rate your dog's behaviour?
Q2[a]	Thinking about your dog's temperament, how would you define its nervousness/restlessness on a scale from 1 to 7 where 1 is 'very nervous and restless' and 7 is 'very calm'?
Q3[a]	Thinking about your dog's general responses, for example, in the presence of unknown people or of new, unknown stimuli (sounds, loud voices, unknown contexts, unknown animals or children…), on a scale from 1 to 7 where 1 is 'extremely fearful and insecure' and 7 is 'very confident', how would you rate your dog?
Q4	Thinking about your dog's behaviour when there is food around, on a scale from 1 to 7 where 1 is not at all aggressive and 7 is very aggressive in the presence of food, how would you rate your dog?
Q5	Some dogs tend to be very insistent and seek physical contact with owners by jumping up, snapping, scratching with a front paw, whining or barking: on a scale from 1 to 7 where 1 is 'no attention seeking behaviours' and 7 is 'frequent and intense attention seeking behaviours', how would you rate your dog?
Q6	Some dogs bark at any time, night and day, some others bark only in exceptional occasions. On a scale from 1 to 7 where 1 is 'rare barking' and 7 is 'frequent and intense barking', how would you rate your dog's behaviour?
Q7	Some dogs are very obedient, for example they come when called and go to bed when asked, while some others are less easily controlled. On a scale from 1 to 7 where 1 is 'not at all obedient' and 7 is 'very obedient', how would you rate your dog's behaviour?
Q8	Some dogs are very predisposed to guarding behaviour and tend to threaten people by barking and growling, some others are friendly with everyone and don't show any guarding behaviour. On a scale from 1 to 7 where 1 is 'no guarding behaviour' and 7 is 'intense & frequent guarding behaviour', how would you define your dog's behaviour?
Q9	Some dogs tend to startle very easy, for example when they hear a sound or are suddenly touched. In these cases they can react by fleeing, getting jumpy or showing aggression. On a scale from 1 to 7 where 1 is 'low/rare startle response' and 7 is an 'excessive and very frequent startle response', how would you define your dog's behaviour?
Q10	Some dogs tend to react aggressively if someone tries to touch them or come close while they are resting. These dogs can become aggressive whenever the owner tries to brush them, medicate them or even simply tries to pet them. On a scale from 1 to 7 where 1 is 'never aggressive when disturbed/restrained' and 7 is 'very aggressive when disturbed/restrained', how would you define your dog's behaviour?
Q11	Some dogs have a marked tendency to avoid people or situations that are unknown or unfamiliar, for example they tend to leave the room when unknown guests arrive or when people scream or there are loud noises. On a scale from 1 to 7 where 1 is 'no tendency to hide or avoid people or situations' and 7 is 'High tendency to hide or avoid people or situations', how would you rate your dog's behaviour?
Q12	Some dogs can show a tendency to mount people (children and adults) or other dogs, often of the same sex. On a scale from 1 to 7 where 1 is 'no tendency to mount' and 7 is 'high tendency to mount' how would you rate your dog's behaviour?

[a] Reversed scale.

Responses were collated and analysed using a repeated measures multivariate GLM (SPSS 21). In this analysis treatment related effects on behaviour when on and off drug were considered as dependent measures; types of treatment (divided into 3 categories: corticosteroids, corticosteroids and other drugs, only drugs other than corticosteroids), duration of treatment (divided into 5 categories: 1 week, 1–2 weeks, 2–3 weeks, 2–4 weeks, more than 4 weeks), the reason for treatment (divided into 3 categories: dermatological conditions, orthopaedic conditions and others) were considered independent factors. This first multivariate analysis was made to test drug effects within-subjects, and since only treatment type was found to be a significant factor and there was great variation in the baseline value of subjects, a univariate GLM was then used to examine the difference between behaviour when on and off treatment versus treatment type, with post hoc comparisons corrected for multiple testing by means of a Bonferroni correction procedure.

2.2. Behavioural test

2.2.1. Subjects

Eleven dogs receiving (or due to receive) corticosteroid treatment and 11 control dogs were recruited and successfully completed two sessions of the behavioural test. Treatment dogs were recruited from the patients of veterinary practices in the North of Italy. Veterinarians were asked to propose dog owners that had received prescriptions of oral corticosteroid drugs for dermatological problems to participate in the research. Criteria for inclusion were that dogs had not been prescribed any other medication; the prescription dose range was within 0.4–0.5 mg/kg of prednisone or methylprednisolone every day. Control dogs were recruited from among the healthy patients of veterinary practices and clients of dog trainers. Control dogs were tested twice in the same environment as the dogs on corticosteroids, with the same time interval between the two tests. Details of all subjects are given in Table 2.

The first behavioural test for treatment dogs occurred just before they started therapy, with a second taking place 6–7 days into the therapy, often just before the dose of corticosteroid started being reduced with a view to its withdrawal.

2.3. Test procedure

The tests were conducted in three different locations in order to accommodate the travel restrictions of clients, but the set-up was the same at each of these: a room of sufficient size to accommodate the experimental apparatus, with a chair for the owner at the opposite end of the room. The apparatus composed of a screen covered with a blanket that hid a loudspeaker system connected to a computer. Five pots were placed in front of the screen, 35 cm from the loudspeakers. The pots were placed in a way that enabled the researcher to put small pieces of food into them at the same time (Fig. 2). For each dog, the kind of treat used during testing was indicated by the owner as being the dog's favourite. The same kind of treat was used in both test trials for that subject. Two video cameras (Canon Legria HF R506) were used to record the dog's behaviour during testing for later

Table 2
Dogs involved in the study. M = male dog; F = female.

Dog n.	Breed/type	Gender	Age	Treatment[a]
1	Collie	M (n)	9	0.5/kg prednisone
2	Dachshund	M	4	0.4/kg prednisone
3	Labrador	M	5	0.4/kg methylprednisolone
4	Golden retriever	F (n)	10	0.5/kg prednisone
5	Crossbreed	F (n)	4	0.4/kg prednisone
6	Cocker	M	9	0.4/kg methylprednisolone
7	Pitbull	M (n)	7	0.4/kg prednisone
8	Jack Russell	M	3	0.5/kg prednisone
9	Crossbreed	M	1	0.4/kg prednisone
10	Golden retriever	F	2	0.5/kg prednisone
11	Crossbreed	M	4	0.4/kg prednisone
12	Crossbreed	M (n)	9	No treatment
13	Crossbreed	F (n)	2	No treatment
14	Crossbreed	M	2	No treatment
15	Pitbull	M (n)	2	No treatment
16	Shiba Inu	M	1	No treatment
17	German shepherd	F	1	No treatment
18	Crossbreed	F (n)	7	No treatment
19	Cocker Spaniel	M	6	No treatment
20	Border collie	M	5	No treatment
21	German shepherd	M	4	No treatment
22	Crossbreed	F (n)	1	No treatment

Dog (n) = neutered.
[a] No dogs were receiving treatment in the first test trial, treatment refers to medication in use during the second test trial.

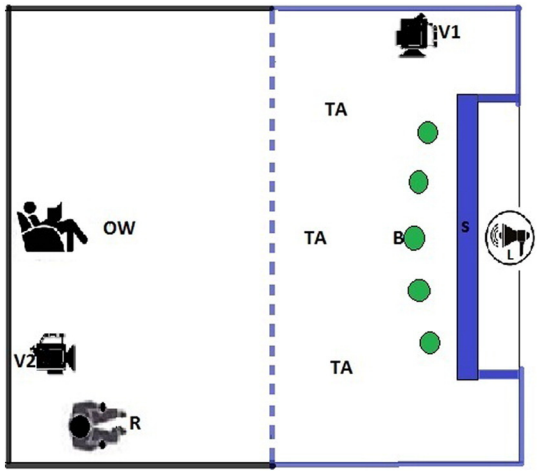

Fig. 2. Test setting. V1 and V2 = video cameras; TA = test area; S = screen;

behavioural coding. The video cameras were mounted on a tripod, one to one side of the room and the other at the back of the room. The part of the room within 150 cm of the screen was considered to be the "test area" and when dogs were within this area with evident interest in exploring it, the screen or the pots, their behaviour was considered as 'exploring the test area'.

Exploring the test area included:

1. Sniffing = The dog overtly approached the floor, the bowls or the screen and appeared to inhale through its nose
2. Exploring = Remaining in the test area watching towards the floor, the screen or the bowls
3. Investigating the pots = approaching the pots with nose within 1 cm of pot and nose or muzzle inside pot

Behaviours such as staying far from the screen, either close to the owner or at the opposite end of the room to the experimental apparatus, were considered as behaviours associated with not exploring the test area.

Dogs were brought into the test environment on a leash by their owners. In each test trial the owner was invited to calmly restrain the dog on the leash, sit and wear a pair of sunglasses to restrict eye contact between dog and their owner. The researcher showed the dog a few pieces of food and then put one small piece of food in each pot. After this, the researcher sat on a chair in a corner of the room, showing no overt interest in the procedure. The owner was instructed to unleash the dog and then behave in a neutral way, pretending to read a book provided by the researcher and completely ignoring the dog until a signal signifying the end of the test was given. The dog was left free to investigate the test area and take the treats from two pots. As soon as it started to approach the third pot, playback of a three second growl was started. Three types of dog growl recordings were used: small dog, medium dog and large dog growls, and these were allocated on the basis of the size of the dogs being tested (i.e. small test dog = small dog growl) in order to minimize the scaring effect of the growl. The growls were chosen because they had been recorded in the context of food guarding and used in a previous study [25]. The dog's behaviour was then observed for two minutes. At the end of the test, the owner was asked to call the dog and put it on its leash. This behavioural test procedure is illustrated in Fig. 3.

B = pot disposition; L = loudspeaker; OW = owner position; R = researcher position during the test.

2.4. Behavioural observation

The video recordings of tests were analysed using Solomon Coder (http://solomoncoder.com/). We considered the following behaviours for analysis:

1. Latency
   a. Time from release to the approach to the first pot (nose within 1 cm of pot) (Latency 1)
   b. Time from the growl/startle reaction to further investigation of pots (Latency 2)

2. Time spent investigating the test area (TTA)
3. Time spent investigating the pots (TTP)
4. Time spent investigating the test area before the growl (Expl1)

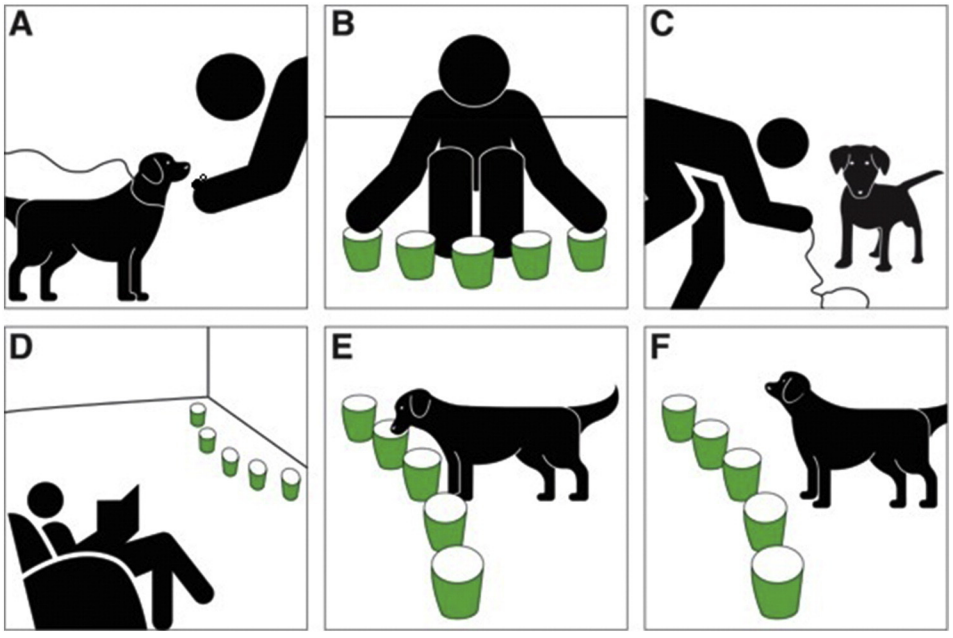

Fig. 3. Behavioural test procedure. A = the dog is shown the food; B = a piece of food is placed into each pot; C = the owner unleashes the dog; D = the owner is instructed to behave in a neutral way; E = the dog explores the pots; F = the dog reacts to the growl.

5. Time spent investigating the test area after the growl (Expl2)
6. Startle reactions
   a. Grade 1. The dog responds with minimal, momentary re-orientation of head
   b. Grade 2. The dog responds with re-orientation of head, steps back
   c. Grade 3. The dog responds with re-orientation of head, steps back and takes a few seconds before coming back to the pot or leaves the test area and does not return within 2 min
7. Eating of food. The number of food pieces eaten by each dog in each test trial.

2.5. Data analysis

The first observer transcribed the video recordings of both test trials (n = 22) and scored them using the ethogram on two separate occasions to assess intra-observer reliability. The recordings from ten of the dogs (5 treatment dogs and 5 control dogs) were randomly selected for their behaviour to be assessed by a second observer who was 'blind' to the treatment allocation in order to evaluate inter-observer variability. Spearman's coefficient was used to measure pairwise correlation among raters.

Data from the video analysis regarding Latency 1, Latency 2, TTP, TTV, Expl1 and Expl2 were analysed using SPSS 21. Data were not normally distributed and therefore an extension of the Generalized Linear Model (GZLM), Generalized Estimating Equations (GEE) was used in order to evaluate the results that accommodated correlated within-subjects data and allowed comparisons between subjects.

Startle reactions were evaluated for their severity according to the above descriptions. Eating of food was evaluated by counting the number of food pieces eaten by each dog during each test trial. Comparison between the control and treatment dogs for these two metrics was evaluated using Mann–Whitney U test at a given time point (e.g. either first or second test), with Wilcoxon's Matched Pairs Signed-Ranks Test used to compare within groups between tests (first versus second test).

3. Results

3.1. Questionnaire study

By the close of the survey in February 2011, 98 questionnaires had been completed correctly by dog owners and considered suitable for analysis. Dogs were from a variety of breeds and genders, and aged between 1–14 years. Reasons for treatment were dermatological conditions (n = 55), orthopaedic conditions (n = 36) and other kinds of condition (n = 7). Treatment duration varied from one week of treatment to long term maintenance treatment. Of the sample of 98 dogs, 44 received corticosteroids and 54 received only other medications, mainly antibiotics (n = 20) and non-steroidal anti-inflammatory drugs (n = 28), with a small proportion on other drugs (n = 6). Of the 44 dogs that received corticosteroids, 23 also received other drugs, mainly antibiotics. The 44 dogs receiving treatment with corticosteroids were subject to a variety of corticosteroid preparations, but mainly prednisone/prednisolone (n = 32) and methylprednisolone (n = 7). Two dogs received betamethasone and 3 dogs received dexamethasone. Corticosteroid drug doses were between 0.1–1.2 mg/kg for prednisone and prednisolone, between 0.5–1.5 mg/kg for methylprednisolone, 0.05 mg/kg for betamethasone and 0.1 mg/kg for dexamethasone.

Data were not normally distributed, but still suitable for analysis of variance [26,27]. The repeated measures multivariate GLM analysis showed that the only significant factor related to a change in the behaviour of dogs on and off corticosteroids was the treatment used.

The univariate GLM with post hoc correction for multiple testing showed that the administration of treatments involving corticosteroid (44 dogs) had a statistically significant effect on the response to eight items. Five behaviours, Play ($F = 6.525$, Nervousness ($F = 6.130$), Fear ($F = 13.112$), Startle reactions ($F = 5.705$), Irritable aggression ($F = 5.080$) - all significantly changed with $p < 0.01$; three behaviours, Food aggression ($F = 4.793$), Barking ($F = 4.330$), Avoidance ($F = 4.463$) — all significantly changed with $p < 0.05$). By contrast, treatments without corticosteroids (54 dogs), produced no significant changes ($p > 0.05$) in response to any item and no significant changes in behaviour were related to other drugs (Table 3).

3.2. Behavioural test

The behavioural testing of dogs (11 sample dogs and 11 control dogs) ended in October 2013.

Spearman's coefficient of correlation revealed statistically significant positive correlations between intra-observer (n = 22) and inter-observer (n = 10) measurements. Intraobserver correlations were positive with $r = 0.994$ and $p < 0.01$. Interobserver correlations were positive with $r = 0.996$ and $p < 0.01$ for all items.

GEE revealed no significant differences in Latency1, Latency 2, TTA, TTP, EXPL1 and EXPL2 between groups in the first test trial, before the 'treatment' dogs had been placed on corticosteroids. In the second test trial, the total time spent investigating the test area (TTA) was significantly lower in the group of dogs treated with corticosteroids (unstandardized coefficient—$B = 25.309$; $\chi^2(1) = 6.157$; $p < 0.05$) compared with the control group of dogs. In the second test trial, the exploration time after the growl of dogs (EXPL2) in the treatment group was significantly lower ($B = 26.18$; $\chi^2(1) = 6.600$; $p < 0.05$) compared with the same behaviour in the control group of dogs. Latency times (L1 and L2), time spent investigating the area before the growl (EXPL1) and the time spent investigating the pots (TTP) were not significantly different between the two groups ($p > 0.05$) [Latency 1: $B = -3.573$, $\chi^2(1) = 0.588$, $p = 0.443$; Latency 2: $B = -9.709$, $\chi^2(1) = 0.477$, $p = 0.490$; EXPL1: $B = -0.545$, $\chi^2(1) = 0.310$, $p = 0.577$; TTP: $B = 5.991$, $\chi^2(1) = 2.583$, $p = 0.108$].

Startle reactions in the first test trial were present in six dogs from the treatment group and nine dogs from the control group. Three dogs from the test group were graded at level 1 (S1) and four at level 2 (S2). Eight dogs from the control group were scored at level 1 (S1) and one at level 3 (S3).

In the second test trial seven dogs from the treatment group and nine dogs from the control group produced startle reactions (see Table 4). No significant differences between groups were found as far as startle reactions were concerned ($p > 0.05$).

In the first test trial, seven dogs from the treatment group ate all five food treats, two dogs did not eat any food and two dogs ate three pieces. In the first test trial, eight dogs from the control group ate all the food, two dogs did not eat any food and one dog ate three pieces. In the second test trial, five dogs from the treatment group ate all the food, two dogs ate four pieces, two dogs ate three pieces and two dogs did not eat any food. In the second test trial, all dogs from the control group ate all the food. A Mann–Whitney U test revealed a significant difference between groups as far as number of pieces of food eaten was concerned ($z = -2.765$; $p = 0.028$) with control dogs eating more than treatment dogs. Wilcoxon's Matched Pairs Signed-Ranks Test revealed no significant differences within groups in the two test trials ($p > 0.05$) for either startle or food consumption.

4. Discussion

The results reported here from both studies are consistent with each other and the preliminary findings of Notari and Mills [9] reported previously. This latter study had the main goal of providing information about the possibility that dogs receiving treatment with corticosteroids might show behavioural analogous to those reported in humans and was the starting point for the development of the present study. The survey reported here was a development of the previous methodology,

Table 3
Reported changes in behaviour score on and off different treatments. Scales 1–7 represent the expression of the behaviour. Questions about nervousness and fear had reversed scales.

Response item		CG Off	CG On	OG Off	OG On
Play**	Mean	4.80	4.05	3.80	4.63
	(±SD)	(1.82)	(1.71)	(1.90)	(1.85)
Nervousness**	Mean	4.57	3.75	4.69	5.02
	(±SD)	(1.56)	(1.92)	(1.78)	(1.55)
Fear**	Mean	4.89	3.95	4.54	4.94
	(±SD)	(1.48)	(1.72)	(1.61)	(1.37)
Food aggression*	Mean	2.00	2.57	2.20	2.07
	(±SD)	(1.45)	(2.11)	(1.56)	(1.37)
Attention seeking	Mean	3.91	4.14	3.93	4.15
	(±SD)	(1.70)	(1.84)	(1.86)	(1.77)
Barking*	Mean	2.73	3.43	2.74	2.67
	(±SD)	(1.69)	(2.05)	(1.75)	(1.78)
Obedience	Mean	5.18	4.91	5.07	5.04
	(±SD)	(1.48)	(1.60)	(1.33)	(1.29)
Guarding	Mean	3.39	3.50	3.20	3.31
	(±SD)	(1.87)	(2.05)	(2.10)	(1.92)
Startle reactions**	Mean	2.84	3.77	3.19	3.06
	(±SD)	(1.68)	(2.07)	(1.83)	(1.62)
Irritable aggression**	Mean	1.93	2.43	2.07	1.96
	(±SD)	(1.47)	(1.83)	(1.46)	(1.30)
Avoidance*	Mean	2.27	2.73	2.19	2.11
	(±SD)	(1.80)	(2.05)	(1.48)	(1.42)
Mounting	Mean	1.80	1.75	2.02	1.80
	(±SD)	(1.29)	(1.48)	(1.56)	(1.19)

CG = corticosteroid group; OG = other group. Significant differences between groups calculated with GLM univariate analysis indicated thus * = $p < 0.05$; ** = $p < 0.01$.

using a simple scoring system to allow the harvesting of a larger data set and the inclusion of a control group. All of the behaviours that were reported to change under the influence of corticosteroid drugs in the previous study [9] were found to change significantly in the present study, adding weight to the reliability of these reported effects. Dogs under corticosteroids were reported to be more nervous/restless, more fearful/less confident, more aggressive in the presence of food, barked more, more prone to startle, more prone to react aggressively when disturbed, and more prone to avoiding people or unusual situations. All these findings indicated these drugs might bias sensitivity towards aversion in dogs.

In addition, one further item, not previously reported, but also possibly influenced by changes in affect i.e. amount of play behaviour, was reported to be reduced under corticosteroid treatment and elevated highly under treatment with other drugs (Table 3). The additional discovery of a disparate effect on play between the two classes of pharmacological intervention could be important as the occurrence of play is considered to be a useful indicator of animal welfare, with animals reducing play when they become distressed [21,28,29]. It therefore seems that a reduction in play would be consistent with the potential negative effects of corticosteroids, but it is worth noting that there was a large increase in response to treatment with the other drugs (Table 3), and this may be where the main effect lies, i.e. an elevation in positive mood when these other interventions are used. The finding is all the more interesting as corticosteroids are widely used for their potent anti-inflammatory effects and it might be predicted that their value in relieving pain and irritation means that their use would increase playfulness as a result. However, these results suggest that their negative effects on affective state might mitigate against the predicted positive behavioural effects. It might be that corticosteroids serve to largely increase arousal rather than induce a positive affective state per se, as might be often assumed.

Four other behaviours were inserted as fillers to prevent psychological bias [24]. These fillers where selected among the behaviours that most frequently cause complaints by dog owners [22,23], that we did not expect to be effected by the use of corticosteroids. The robustness of the effects reported here are therefore further enhanced by the finding that these four items (attention seeking, obedience, guarding and mounting) did not show significant effects. Corticosteroid drug dose effect and the effects of disease on behaviour have been widely reported in humans [1,30,31], and the impact of physiological stress and health on the behaviour of veterinary species recently reviewed [32], but there is still a lack of information on the relationship between levels of circulating corticosteroid and behaviour in animals. This was not addressed in the present study but is an area for future attention.

Behavioural tests followed the survey in order to provide, for the first time, objective behavioural evidence of the effect of corticosteroid therapy on dog behaviour. Because our initial findings were interpreted to indicate that dogs on corticosteroid therapy were more avoidant, the

Table 4
Startle reactions and pieces of food eaten by dogs in the two test trials.

Dog n.	Trial 1 Startle score	Trial 2 Startle score	Trial 1 Food eaten	Trial 2 Food eaten
1[a]	–	–	5	5
2[a]	S1	S2	5	5
3[a]	–	–	5	5
4[a]	–	–	5	4
5[a]	S2	S3	3	3
6[a]	–	S2	5	0
7[a]	S2	S1	0	4
8[a]	S2	S2	0	0
9[a]	S1	S1	3	3
10[a]	S2	S2	5	5
11[a]	–	–	5	5
12	S1	S1	5	5
13	–	–	5	5
14	S1	S1	5	5
15	S1	S1	5	5
16	S1	S2	0	5
17	S1	S1	5	5
18	S3	S3	3	5
19	–	–	5	5
20	S1	S1	5	5
21	S1	S1	0	5
22	S1	S1	5	5

S1 = The dog responds with minimal, momentary re-orientation of head.
S2 = The dog responds with re-orientation of head, steps back.
S3 = The dog responds with re-orientation of head, steps back and it takes a few seconds before coming back to the pot or never comes back.
Trial 1 Food = pieces of food eaten in trial 1.
Trial 2 Food = pieces of food eaten in trial 2.
[a] Dogs receiving treatment with corticosteroids in the second test trial.

test was designed to stimulate exploration of the test area with minimal challenges. The introduction of a surprising, potentially negative stimulus in the form of the growl, had the purpose of testing both reactivity and avoidance tendency. Decreases in exploratory behaviour have also been associated with negative affective states [33–35], and this is likely to be the product of a negative cognitive bias associated with negative affect: when in a negative affective state the desire to seek new information is reduced and so the animal might avoid rather than explore open areas and novel stimuli [36,37]. In the behavioural tests, types of corticosteroids and drug doses were very similar within the treatment group. Although cytokines involved in the immune response might explain behavioural effects such as decreased exploratory behaviour and increased avoidance [38] reported by owners in the survey, this would not explain the results in the behavioural tests. Some dogs suffered from allergic dermatological conditions, that would produce a lot of cytokines, when they were tested off treatment and no significant differences were found between treatment and control group dogs at this time rather a significant decrease in exploratory behaviour between groups was observed only when treatment dogs were on corticosteroids.

Unlike the dogs receiving corticosteroid treatment, control dogs did not show a significant change in their exploratory behaviour, when receiving treatment. Comparisons between groups showed that the exploratory behaviour was lower when dogs were on corticosteroids. In laboratory animals, the administration of glucocorticoids after a training session seemed to influence contextual fear memories and hippocampal long term potentiation (LTP), suggesting that they may enhance contextual fear memory consolidation via enhancing hippocampal LTP [39].

In the behavioural test, we found dogs on corticosteroids ate significantly less food compared with the control group, despite increased appetite being a well-recognized side effect of corticosteroid therapy. Decreased food intake can be interpreted as part of decreased exploratory behaviour or as a sign of increased stress in itself [40].

In the behavioural tests three dogs treated with corticosteroids increased their startle reactions and one dog decreased this reaction, while in the control group just one dog increased its startle reaction and no dog decreased it., The results about startle reactions did not show any significant differences in startle reactions between the two groups ($p > 0.05$). However, in the questionnaire study dog owners reported an increase in their dogs' startling tendency when on corticosteroids and the startle response has been used as a specific response to assess a changed negative affective state in both laboratory animals and human beings [3]. This apparent discrepancy can be explained because startle responses have different motor features according to whether they are triggered by emotional or voluntary responses [41–46], and it may be that the two contexts focus on different types of startle.

Direct testing of dogs in the behavioural test may be more reliable, but is more labour intensive, especially when relying on clinical cases. The need to design a behavioural test without compromising animal welfare meant that the chosen stimuli were not so intense as to scare or seriously threaten the animal. As a consequence, the magnitude of the behavioural responses that we observed may have been less compared to reactions that the animal in treatment might have exhibited in real life fearful or threatening conditions. This could explain why the effects in the test seemed relatively mild when compared to the survey results. The findings of the surveys and the tests provide convergent validity that, together with a consistency at the theoretical level, indicate that these results are robust and the effects reliable.

5. Conclusion

Overall, these results indicate that in pet dogs, corticosteroid treatment at therapeutic doses can bias cognition and change behaviour. Physiological intervention with these drugs appears to increase sensitivity towards aversion. On the basis of these results and in the absence of evidence to the contrary, we recommend that the supply of these drugs to owners by veterinarians should be accompanied by advice about behavioural risk management due to a possible increase of a negative affective state, a condition that might increase the risk of aggressive behaviours.

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