Article

Placebo-controlled double-blind clomipramine trial for the treatment of anxiety or fear in beagles during ground transport

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Abstract — The purpose of this explorative study was, first, to document changes in physiological parameters and behavior observed in dogs following ground transport and, second, to measure the effects on the above variables of a short-term administration of clomipramine, anecdotally already prescribed in private veterinary practice to reduce fear, anxiety, or both. Twenty-four beagles were randomly allocated to either clomipramine (2 mg/kg, q12h for 7 d) or placebo treatment, and then transported 3 times in a truck for 1 hour. Physiological parameters (cortisol, neutrophil:lymphocyte (N:L) ratio, heart rate) and behavior were recorded and analyzed. Clomipramine significantly reduced plasma cortisol ($P < 0.05$) following transport and tended ($P = 0.07$) to reduce N:L ratio. Clomipramine tended to only reduce "moving and panting" and drooling. Short-term administration of clomipramine appears to slightly reduce fear, anxiety, or both during transport. More research is needed to confirm the efficacy of this treatment and the appropriate dosage.

Résumé — Étude de la clomipramine en double insu contre un placebo pour le traitement de l'anxiété ou de la peur chez des beagles au cours de transports terrestres. Le but de cette étude exploratoire était d'abord de mesurer les variations des paramètres physiologiques et comportementaux observés chez les chiens à la suite de transport terrestre puis de mesurer les effets sur les variables précédentes d'une administration à court terme de clomipramine, réputée réduire la peur, l'anxiété ou les deux et déjà utilisée en pratique vétérinaire privée. Vingt-quatre beagles ont été alloués au hasard soit au groupe clomipramine (2 mg/Kg, q 12 h pendant 7 jours) soit au groupe placebo puis transportés 3 fois pendant 1 heure dans un camion. Les paramètres physiologiques (cortisol, rapport neutrophile : lymphocyte (N :L) et fréquence cardiaque) et le comportement ont été mesurés et analysés. La clomipramine réduit significativement le cortisol sanguin ($P < 0,05$) à la suite du transport et tend ($P = 0,07$) à réduire le rapport N :L. La clomipramine tendait uniquement à réduire l'agitation et le halètement ainsi que l'hypersalivation. L'administration de clomipramine sur une courte période semble réduire légèrement la peur, l'anxiété ou les deux au cours du transport. D'autres recherches sont nécessaires pour confirmer l'efficacité du traitement et la posologie appropriée.

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Introduction

Transport has been shown to be stressful for many domestic animal species, including cattle, pigs, poultry, sheep (1), horses (2,3), and dogs (4,5). Moberg (6) reported "because the term 'stress' has been used so broadly in biology, no clear definition of stress has emerged." He defines stress as the biological response elicited when an individual perceives a threat to its homeostasis. Traditionally, researchers have relied on a variety of endocrine, behavioral, autonomic nervous system, and immunological assays to measure stress. Some authors measuring stress tend to use different assays together (1,2,5,7–15). Stress response may result from either fear or anxiety (16). Fear is defined as an emotional response to a potentially harmful stimulus, whereas anxiety is the emotional response to a stimulus that predicts a potentially harmful or unpredictable environment (16). Hence, anxiety is the anticipation of danger, whether real or imaginary. For companion animals such as dogs and cats, little research has been published on stress, anxiety, or both during car travel. Some cats and dogs require sedation for car travel and, traditionally, veterinarians have prescribed acepromazine, a phenothiazine derivative. Acepromazine is labelled for use in dogs, cats, and horses and is used regularly for its tranquilizing

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action. Phenothiazines are not the drugs of choice to treat fearful or phobic behaviors, because they have poor anxiolytic activity and produce marked sedation (17). Physiological measures of stress were unchanged in dogs receiving acepromazine during air transport (7). Phenothiazine tranquilizers blunt normal and abnormal behaviors and some dogs become more reactive to noise (18–20). Effects such as duration and level of tranquilization may be individually variable and may be breed dependent (17).

If dogs and cats are in fact stressed during car transport, perhaps medications other than phenothiazines might be more effective at reducing fear or anxiety. Some veterinarians in France anecdotally report using either tricyclic antidepressants, such as clomipramine, or selective serotonin reuptake inhibitors, such as fluoxetine or fluvoxamine, for traveling dogs or cats. The purpose of this explorative study was 2-fold: First, to document changes in physiological parameters (cortisol, neutrophil: lymphocyte ratio, heart rate) and behaviors observed in dogs during ground transport and, second, to document whether changes in physiological parameters or behaviors were observable following short-term administration of clomipramine, since this is a treatment anecdotally already prescribed in private practice.

Materials and methods

Animals

Twenty-four beagles from the colony belonging to the animal care facility of the veterinary school (Faculté de Médecine Vétérinaire de l'Université de Montréal) were selected. Twelve entire males, average age 18 mo (range 16–20 mo), weighing 13.49 kg (range 11.2–16.6 kg) and 12 entire females, average age 23 mo (range 16–30 mo), weighing 11.28 kg (range 8.4–13.2 kg), were included in the study. The beagles were housed singly for 4 d, namely after transport and 3 additional days for a boarding study. Blood sampling for cortisol measures was done pre- and post-transport before they were housed singly. Then, the beagles were group housed again for the "wash-out" week prior to the next transport. Study inclusion criteria required normal findings on physical examination, as well as complete blood (cell) counts (CBC) and blood chemical analyses within normal limits. Exclusion criteria included any physical or blood abnormality, as well as uncooperative behavior during blood sampling techniques. Three dogs were excluded (2: blood abnormalities; 1: struggling with minimal restraint) and replaced by qualifying ones. Food was provided at 0800 and water was available ad libitum. Administration of clomipramine with or without food is believed not to produce clinically important differences in efficacy (21).

Treatments

The study protocol followed Canadian Council on Animal Care (CCAC) guidelines and was approved by the Animal Care Committee of the Faculty of Veterinary Medicine. Throughout this placebo-controlled, double blind study, dogs were allocated to 1 of 8 groups: either 1 of 4 groups [M1 to M4] of 3 males/group or 1 of 4 groups [F1 to F4] of 3 females/group. Each group was transported 3 times, and treatments were alternated between transport episodes according to a switchback design, in which the 2 groups of the same sex received treatments (placebo or clomipramine) in the opposite order (Table 1). Treatment allocation was done to ensure that each truckload contained an equal number of animals (3) per treatment order. The dogs were transported in a truck in which 6 kennels measuring 0.58 m long by 0.40 m wide by 0.47 m high could be secured. Groups M1, M2, F1, F2 always traveled in the morning and groups (M3, M4, F3, F4) always traveled in the afternoon (Table 1). Females traveled on the same day (morning or afternoon), and alternated weekly with transportation of the males. All animals had prior experience of ground travel, although duration of transportation may have differed from one dog to another. Space allocation in the truck was assigned "randomly." Each group traveled every other week for an hour. The driver (veterinary student) and the travel circuit were the same for all 6 days. Clomipramine (Clomicalm®; Novartis Animal Health, Basel, Switzerland) in Canada is labelled for use in the dog at a dosage of 1–2 mg/kg body weight (BW) twice daily for the treatment of separation anxiety and obsessive-compulsive disorders. Drug or placebo dose was set at 2 mg/kg BW, q12h. Actual dose for females ranged from 1.8 to 2.3 mg/kg BW and 1.8 to 2.2 mg/kg BW for males. Tablet administration was done daily at 0800 and 2000 for a week: 3 d prior to truck travel, on the day of travel, and for 3 d post-travel (boarding study). The aim in this particular study was to reach a steady state of the medication by the time dogs were scheduled to travel by truck. Published pharmacokinetic studies of clomipramine in the dog indicate that steady state is reached within 4 d (22) or less. Travel day, therefore, corresponded to the 4th day on medication or placebo. A dose of 2 mg/kg BW, q12h daily was also selected for initial pharmacological treatment of storm phobia in dogs (23). Following transportation, dogs were boarded in individual cages for 3 days, during which they continued to receive either placebo or medication. Medication was discontinued without a weaning process. Plasma half-life of clomipramine in dogs after a single dose (1 mg/kg BW, q24h; 2 mg/kg BW, q24h; 4 mg/kg BW, q24h) is reported to be 1.9 h (range 1.0–3.8 h) and 2.8 h (range 1.6–5.0 h) after repeated dose administration (22). Hewson et al (24), on the other hand, reported that the half-life of clomipramine in dogs after a single oral dose (3 mg/kg BW, q24h) ranged from 1.2 h to 16 h. Following multiple oral doses, the half-life ranged from 1.5 h to 9 h. The half-life for desmethylclomipramine in dogs following a single oral dose of clomipramine

Table 1. Treatment and travel design

		Dogs travelling together	
Week 1	AM	3 F1 placebo	3 F2 clomipramine
	PM	3 F3 placebo	3 F4 clomipramine
Week 2	AM	3 M1 placebo	3 M2 clomipramine
	PM	3 M3 placebo	3 M4 clomipramine
Week 3	AM	3 F1 clomipramine	3 F2 placebo
	PM	3 F3 clomipramine	3 F4 placebo
Week 4	AM	3 M1 clomipramine	3 M2 placebo
	PM	3 M3 clomipramine	3 M4 placebo
Week 5	AM	3 F1 placebo	3 F2 clomipramine
	PM	3 F3 placebo	3 F4 clomipramine
Week 6	AM	3 M1 placebo	3 M2 clomipramine
	PM	3 M3 placebo	3 M4 clomipramine

Table 2. Treatment effect on plasma cortisol concentration and neutrophil: lymphocyte (N:L) ratio of dogs before and after ground transport

	Placebo		Clomipramine		
Variables	Mean	Standard Deviation	Mean	Standard Deviation	*P*-value
Plasma cortisol Departure (nmol/L)	36.19	19.38	41.7	20.09	NS
Plasma cortisol Return (nmol/L)	239.64	90.37	224.55	109.97	0.05
Departure-return difference	203.42	95.29	182.84	119.4	0.03
N:L Ratio — Departure	3.36	1.42	3.19	1.3	NS
N:L Ratio — Return	6.32	3.39	5.13	2.47	0.07 T
Departure-return difference	2.95	2.92	2.34	3.19	NS

(3 mg/kg BW, q24h) ranged from 0.66 h to 2.3 h, whereas following multiple oral doses it ranged from 1.4 h to 4.3 h (24). To avoid differences in clinical effects of the medication between the 1st and subsequent trips, a 7-day washout period (25) was used between trips. Usually a departure for morning travel took place between 0945 and 1030 and for afternoon travel between 1415 and 1500. One week preceding the 1st truck ride, the skin over both cephalic veins was shaved for venipuncture and blood samples were taken for the initial CBC and biochemical analyses. Hair on the left and right sides of the thorax was also clipped in preparation for future heart rate monitoring. Subsequently, hair was clipped as needed over the thorax and veins during the week preceding the next truck ride.

Physiological measures

Blood sampling was done prior to and following truck transportation to measure plasma cortisol levels and white blood cell counts to assess N:L ratios. Starting on average 50 min before transportation, dogs were placed on a table where they either sat down or stood. They were restrained minimally while blood was collected in 3-mL EDTA tubes and in 3-mL tubes with a blood collection set (23G3/4, Vacutainer®; Becton Dickinson, Franklin Lakes, New Jersey, USA). The same student took all of the blood samples and was successful on 1st attempt for 71 of the 72 samples, only on 1 occasion requiring a 2nd venipuncture. White blood cell counts were done on the same day as collection. All blood samples for cortisol assays were centrifuged within 1 h following collection and the plasma was frozen at –70°C until being analyzed. All samples were analyzed on the same day, 1 mo following the end of the study. Plasma cortisol determinations were obtained by radioimmunoassay, using a commercial kit ("Coat-A-Count"; Diagnostic Products, Los Angeles, California, USA). Sensitivity of the method and coefficient of variation reported by the supplier were 5.5 nmol/L and 6.3%, respectively.

Prior to and following each trip and blood sampling, rectal body temperature was taken. Because travel took place during summer months when daily temperatures can reach 30°C or higher, temperature and relative humidity within the vehicle cabin were measured at departure and return times for each truck ride. Temperatures ranged from 22.2°C to 35°C (mean 28.6, s = 3.7) and humidity from 42% to 84% (mean 59, s = 11%). The dogs were fitted with a heart rate monitor (Polar Xtrainer Plus; Polar, Richard Browne & Co., North York, Ontario), which stored data at 5-s intervals. The transmitting belt was covered with an elastic bandage (Vetrap; 3M Company, Montreal, Quebec) to keep it in place. A cotton jacket was placed on the dogs to protect the monitor. Dogs were then transferred to the travel kennel.

Behavioral measures

A battery-powered camera (BCD 468; Vidamax Canada, Laval, Quebec) was mounted on the outside of each kennel to record behavior. Two groups of 3 cameras were each connected to a sequential switcher (SDR4; Vidamax Canada) and a battery-operated video recorder (AG-1070; Panasonic, Secaucus, New Jersey, USA). A scan sampling technique was used for the recording, whereby each dog in a group of 3 was filmed for 20 s. Cameras were activated as soon as the dog was placed in the kennel. The video recorder was turned on just prior to departure.

Behaviors were recorded in terms of frequency or duration of occurrence by a different observer unfamiliar with the study protocol. Postures (lying, sitting, standing) and activities (panting, moving, moving and panting, immobile, vomiting, biting the vest, biting or tearing the paper, biting the cage, circling) recorded as "state" were reported as percentage of observation time, and "events" (licking lips, wagging tail, yawning, vocalizing, licking the cage, and drooling) were reported in terms of frequency of occurrence.

Statistical analysis

Treatment effects were analyzed by using the general linear model (GLM) procedure of a statistical analysis system (SAS) (26), according to a switchback design (27). When data were not normal, transformations were done. A repeated measure analysis was performed on physiological data with the GLM procedure of the SAS (26), to compare pre- and post-transport values.

Results

Physiological measures

Plasma cortisol levels

Ground transport caused an increase in cortisol regardless of treatment received (Table 2). However, the increase was significantly lower ($P < 0.05$) in animals treated with clomipramine than in animals receiving placebo. Cortisol levels pre-transport were higher in dogs receiving clomipramine than in dogs receiving placebo, but this difference was not significant (Table 2).

Table 3. Treatment effect on canine behavior during ground transport

	Placebo		Clomipramine		
Variables	Mean	Standard deviation	Mean	Standard deviation	*P*-value
States (% Observation time)					
Lying down	12.95	18.97	21.97	27.99	NS
Sitting	81.44	20.22	74.81	26.82	NS
Standing	5.59	7.93	3.26	3.83	NS
Panting	42.46	27.13	36.28	26.95	NS
Moving	24.76	17.97	22.17	10.58	NS
Moving, panting	8.30	8.48	5.74	7.41	0.10 T
Immobile	18.23	19.64	28.87	23.1	NS
Events (Occurrences per 20 min)					
Licking lips	82.78	57.16	69.45	44.31	NS
Tail wagging	0.25	0.7	0.33	0.73	NS
Yawning	2.39	2.07	2.48	2.65	NS
Vocalisation	31.60	79.76	11.44	35.99	NS
Licking cage	0.17	0.94	0.07	0.38	NS
Drooling	10.75	10.16	9.29	10.41	0.06 T

T = tendency
NS = Not significant

Neutrophil:lymphocyte ratios

Dogs receiving clomipramine tended ($P = 0.07$) to show lower neutrophil:lymphocyte ratios after transport when compared with dogs receiving placebo. The pre-transport N:L ratio and the increase following transport (post-transport minus pre-transport) were lower in dogs receiving clomipramine compared with control dogs, but the difference between groups was not significant (Table 2).

Heart rate

Average heart rate beats/min (bpm) during ground transport was significantly ($P < 0.005$) lower in dogs receiving clomipramine (117.3 bpm) compared with dogs receiving placebo (125.6 bpm). Peaks of 189 bpm were observed during transport.

Behavioral measures

No significant differences were noted for the postures standing, lying, or sitting. The dogs spent most of their time (75% to 80%) sitting. Even though there was no significant difference, time spent lying down by dogs receiving clomipramine was double that in dogs receiving placebo. Time spent moving and panting tended to be less for dogs receiving clomipramine ($P = 0.10$). No significant difference was noted in terms of activities between the 2 treatment groups, despite apparently large differences between treatment means for some variables (time immobile). Activities recorded in terms of frequencies revealed that dogs on clomipramine tended ($P = 0.06$) not to drool as frequently as dogs on placebo (Table 3). Otherwise, results were not significantly different.

Discussion

Even though we were not always able to show statistical significance with all parameters, there was a general tendency for a decrease in physiological responses associated with stress, as well as a decrease in the frequency or duration of some of the behaviors compatible with anxiety in dogs on clomipramine compared with dogs on placebo. Our study showed a tendency toward increased N:L ratio following ground transport, whereas the study by Beerda et al (5) showed a significant N:L ratio increase in dogs following car transport compared with baseline prior to travel. We observed increased plasma cortisol levels following transportation for both treatment and placebo, similarly to Kuhn et al (4), although our post-transport average values were higher than in Kuhn et al's study. We can therefore conclude that dogs transported are "stressed." In our study, temperature and humidity inside the truck were relatively high (mean 28.6, $s = 3.7$). In Kuhn's study, temperatures within the truck varied between 17°C and 23°C and dogs were transported for 9.5 h. In the Beerda et al (5) study, car transportation lasted 50 min, but information on environmental temperature and humidity are lacking. Transport can bring together different stressors, such as unusual experience, unfamiliar environment, high temperatures, noise, and vibrations. Additionally, some animals may suffer from motion sickness and vomit.

Restraint and venipuncture may cause sufficient stress to increase baseline cortisol levels when sampling is not performed promptly following restraint or if restraint is excessive (14). Knol et al (28) actually compared effects of methods used for blood collection on plasma concentrations of luteinising hormone, testosterone, and cortisol in trained male experimental dogs. They reported that whether blood samples were drawn in a treatment room (unfamiliar environment) or in the kennel (familiar environment) from the cephalic vein or through an indwelling catheter in the jugular (control), plasma levels of the 3 hormones were unaffected. Their conclusion was that the method of blood collection used did not influence the validity of results obtained under their experimental conditions. Normal plasma cortisol levels in dogs vary from 13.79 to 165.54 nmol/L (29). Cortisol is secreted episodically, with bursts of secretion occurring throughout the day, thus creating a myriad of peaks and valleys in the plasma cortisol concentrations. These fluctuations account for the wide reference range for plasma cortisol concentration in dogs (29). According to Thun et al (30), secretion of cortisol and testosterone in intact male dogs occurs without any evidence of circadian rhythmicity or seasonal variation. Kemppainen and Sartin (31) also stated that there was a lack

of circadian rhythmicity for cortisol plasma levels in anoestrous female and intact male crossbred dogs. Our cortisol baseline values compared favorably with baseline values measured in dogs fitted with catheters (28) and with values published elsewhere in the literature (9,14,15), suggesting that our blood sampling technique did not interfere with cortisol secretion.

Clomipramine has been reported to significantly decrease heart rate at a dose of 20 mg/kg BW, PO, q24h, with peak effect achieved approximately 12 h after dosing. No significant effects on heart rate were noted when clomipramine was administered at 4 mg/kg BW and 12 mg/kg BW q24h (25). Additionally, Pouchelon et al (25) concluded that the cardiovascular effects did not correlate directly with plasma concentrations of clomipramine or desmethylclomipramine. They felt that their results were consistent with an indirect or centrally mediated mechanism of action. Most of the anti-anxiety actions of clomipramine are assumed to be the consequence of inhibition of neuronal reuptake of serotonin, and these authors hypothesized that the heart rate lowering effects were achieved via a similar mechanism. Because of the low dose used (2 mg/kg BW, q12h) and the reported lack of correlation between plasma concentration and cardiovascular effects, we could therefore hypothesize that dogs on clomipramine in the present study were less anxious, less stressed, or both, than dogs on placebo.

Fearful animals can become immobile (freeze), run (flee), or fight. The specific appearance of a fearful or anxious animal will vary, but in general the tail is down or tucked, the ears are pinned back against the head, and the eyes are wide with dilated pupils. The animal may yawn or lick its lips repeatedly, may tremble, or may exhibit piloerection (32). When confined, an increase or decrease in the motor activity of a given animal in itself is insufficient to conclude that this animal is stressed or not. Body posture tends to lower with fear, anxiety, or submission. There is a common stress response resulting from either fear or anxiety (16). Beerda et al (5) reported increased performance of tongue out; snout licking; paw lifting; body shaking, along with a lowered body posture; and increased heart rate and saliva cortisol in 1 dog subjected to 95 dB noise. They concluded that these behaviors could be indicative of stress. In another study, Beerda et al (9) found that dogs that were subjected to different types of stimuli (pushing dog down by pressing on the neck and back; pulling head of dog down to ground via a rope/bar system; opening an umbrella; bag filled with paper dropped from the ceiling; noise; electric shock) performed more body shaking, crouching, oral behaviors (tongue out, tip of tongue briefly extended, snout licking, swallowing, smacking), yawning, and restlessness, and presented a low posture. It is unclear if tongue out in that study indicated panting or not. Mouth licking, front paw lifting, ears pulled back, and lowered standing or sitting postures have also been reported as indications of stress in dogs subjected to harsh training methods (33). Dogs trained harshly (physical corrections) also vocalized, whereas dogs trained with rewards did not. Vocalization can occur for various reasons, such as excitement, play, communication, attention seeking, threat, pain, or defence (18). Hetts et al (34) found that dogs housed in the greatest degree of social isolation spent the most time moving, exhibited the greatest number of abnormal movements, and vocalized the most. Vocalization can also occur as a consequence of fear or anxiety (18,35). The nature of the bark usually changes depending on the context in which it is used. The video equipment used in the present study did not record sound, so the nature of the bark could not be qualified. The determination of barking was made on the basis of the behaviors observed.

Our study indicated that dogs spent most of their time sitting in their kennel regardless of treatment. Because of large individual differences, no significant differences could be detected between treatments for most behavioral data. Even though no firm conclusion can be drawn from these results, it is interesting to note that dogs receiving clomipramine appeared to be calmer than control dogs. Simply looking at the means, they were, in general, less active and they did not pant, lick their lips, drool, or vocalize as frequently as dogs on placebo. Dogs being less active could be a consequence of sedation, which is reported as a side effect of clomipramine. However, none of the dogs prior to the truck ride or following the truck ride showed any signs of sedation. Panting can be associated with increased body temperature, fever, anxiety, or fear. All dogs were subjected to the same environmental conditions, yet those on the medication did not pant as frequently. Drooling and licking lips can be associated with nausea, as well as anxiety or fear. Dogs on clomipramine did not vocalize as frequently. Clomipramine is labelled for use in dogs for the treatment of separation anxiety. One of the clinical manifestations of separation anxiety is vocalization during owner absence, and clomipramine has been shown to be very effective in decreasing vocalization in patients suffering from this disorder (36). The signs previously described (increased movement or activity, panting, drooling, licking lips, vocalizing) are nonspecific. However, one could hypothesize that taken together in this context, these signs are the consequence of anxiety/fear and that the dogs on clomipramine were therefore somewhat less anxious. A larger scale study is needed to confirm this hypothesis. As mentioned earlier, our physiological results also suggest that dogs on clomipramine were less stressed and, therefore, possibly less anxious or fearful. Plasma cortisol levels after transport and mean heart rate per minute during transport were lower in dogs on clomipramine compared with control dogs. Interestingly, results published on the physiology and behavior of dogs during air transport showed that tranquilization with acepromazine at the dosage and timing used did not affect physiological and behavioral stress responses (7). The exact mechanism of action of acepromazine is not fully understood, but the phenothiazines in general block post-synaptic dopamine receptors in the central nervous system (CNS) and may also inhibit the release, as well as increase the rate of turnover, of dopamine (37). In dogs, acepromazine's effects include a decrease in blood pressure, as well as bradycardia. Phenothiazines, also called neuroleptics or antipsychotics, have been used nonspecifically to manage behavior problems in animals, such as noise phobia or aggression, by reducing the animal's general attendance to environmental stimuli and by producing sedation (38). They do not decrease anxiety. On the other hand, psychotropic medications that block the reuptake of serotonin may have significant antianxiety effects in humans

(39) and clomipramine has been shown to be effective in at least 1 uncontrolled study. Clomipramine is a tricyclic antidepressant but differs from other tricyclic antidepressants. It is believed that the most significant effects of clomipramine result from its action in preventing the reuptake of norepinephrine and serotonin at the neuronal membrane (40). Clomipramine is a selective inhibitor of serotonin reuptake, whereas its intermediate metabolite desmethylclomipramine is an inhibitor of norepinephrine reuptake. Tricyclic antidepressants (TCAs), such as clomipramine, and selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, fluvoxamine, and paroxetine, are used to treat different human psychiatric conditions. For instance, they can be used to treat depression, generalized anxiety disorder, panic disorder, and obsessive-compulsive disorder (39,41). Recent studies have suggested that a possible mechanism by which antidepressants exert their effect is through direct modulation of the glucocorticoid receptor (42). These same psychotropic medications are also used to treat various behavioral conditions in different species (19,38,43–46), including anxiety-related disorders.

One question raised by this study is whether clomipramine should be recommended as an alternative to acepromazine for treating dog transport anxiety/fear/stress. Measuring welfare during transport requires that we consider the animal's ability to cope with the environmental stresses and the amount of effort required to cope. Physiological and behavioral responses to transport can be used to assess the amount of effort animals must make to cope (47). But caution is urged (48), since, for example, the extent of cortisol rise may not be sufficient to rate the degree of unpleasantness of a given experience. Stress is complex and individual specific (49). During periods of adaptation, there is little question that animals are under some degree of stress. Stress is, in fact, an essential stimulus for learning and adapting to new situations. Stress responses have evolved to deal with or adapt to particular stressors, which means that the type of behavior shown will probably be context specific (50). Stress behaviors should, therefore, be interpreted in combination with physiological data and within their context of occurrence (51). The decision for a veterinarian to medicate a given dog during car transport should be based on that animal's behaviors, as well as its inability to adapt to car transport (the dog is not improving or is getting worse with each additional car travel). Clomipramine is currently labelled for use in dogs in Canada for the treatment of separation anxiety and obsessive-compulsive disorders. Although not labelled for car travel, it could potentially be an appropriate drug choice for this use, since it did show a tendency to decrease behavioral signs compatible with anxiety/fear and physiological measures compatible with stress. Additional studies, using various different dosages of clomipramine, as well as comparing truly punctual versus regular daily use of the medication, would be useful.

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References

1. Knowles TG, Brown SN, Wariss PD, et al. Effects on sheep of transport by road for up to 24 hours. Vet Rec 1995;136:431–438.
2. Smith BL, Jones JH, Hornof WJ, et al. Effects of road transport on indices of stress in horses. Equine Vet J 1996;28:446–454.
3. Friend TH, Martin MT, Householder DD, Bushong DM. Stress responses of horses during a long period of transport in a commercial truck. J Am Vet Med Assoc 1998;212:838–844.
4. Kuhn G, Lichtwald K, Hardegg W, Abel HH. The effect of transportation stress on circulating corticosteroids, enzyme activities and hematological values in laboratory dogs. J Exp Anim Sci 1991;34:99–104.
5. Beerda B, Schilder MBH, Van Hooff JA, de Vries HW. Manifestations of chronic and acute stress in dogs. Appl Anim Behav Sci 1997;52: 307–319.
6. Moberg GP. Biological response to stress: Implications for animal welfare. In: Moberg GP, Mench JA, eds. The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Wallingford, UK: CABI Publ, 2000:1–21.
7. Bergeron R, Shannon L, Émond JP, et al. Physiology and behavior of dogs during air transport. Can J Vet Res 2002;66:211–216.
8. Beerda B, Schilder MB, Janssen NS, Mol JA. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for noninvasive assessment of stress responses in dogs. Horm Behav 1996;30: 272–279.
9. Beerda B, Schilder MBH, Van Hooff JA, de Vries HW. Behavioral, saliva cortisol and heart rate responses to different types of stimuli in dogs. Appl Anim Behav Sci 1998;58:365–381.
10. Beerda B, Schilder MB, Van Hooff JA, de Vries HW, Mol JA. Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. Physiol Behav 1999;66:243–254.
11. Beerda B, Schilder MB, Bernadina W, Van Hooff JA, de Vries HW, Mol JA. Chronic stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological responses. Physiol Behav 1999; 66:233–242.
12. Beerda B, Schilder MBH, Van Hoff JA, De Vries HW, Mol JA. Behavioral and hormonal indicators of enduring environmental stress in dogs. Anim Welfare 2000;9:49–62.
13. Clark JD, Rager DR, Crowell-Davis S, Evans DL. Housing and exercise of dogs: Effects on behavior, immune function, and cortisol concentration. Lab Anim Sci 1997;47:500–510.
14. Hennessy MB, Williams MT, Miller DD, Douglas CW, Voith VL. Influence of male and female petters on plasma cortisol and behavior: can human interaction reduce the stress of dogs in a public animal shelter? Appl Anim Behav Sci 1998;61:63–77.
15. Hennessy MB, Voith VL, Mazzei SJ, Buttram J, Miller DD, Linden F. Behavior and cortisol levels in dogs in a public animal shelter, and an exploration of the ability of these measures to predict behavior problem after adoption. Appl Anim Behav Sci 2001;73:217–233.
16. Casey R. Fear and stress in companion animals. In: Horwitz D, Mills D, Heath S, eds. BSAVA Manual of Canine and Feline Behavioral Medicine. Gloucester, UK: Br Small Anim Vet Assoc, 2002: 144–153.
17. Thompson SB. Pharmacologic treatment of phobias. In: Dodman NH, Shuster L, eds. Psychopharmacology of Animal Behavior Disorders. Malden, Massachussets: Blackwell Science, 1998:141–182.
18. Landsberg GM, Hunthausen W, Ackerman L. Handbook of Behavior Problems of the Cat and Dog, 2nd ed. London: Saunders, 2003: 126–128 and 314.
19. Overall KL. Pharmacologic treatments for behavior problems. Vet Clin North Am: Small Anim Pract 1997;27:637–665.
20. Overall KL. Noise phobia in dogs. In: Horwitz D, Mills D, Heath S, eds. BSAVA Manual of Canine and Feline Behavioral Medicine. Gloucester, UK: Br Small Anim Vet Assoc, 2002:164–172.
21. King JN, Maurer MP, Hotz RP, Fisch RD. Pharmacokinetics of clomipramine in dogs following single-dose and oral dose administration. Am J Vet Res 2000;61:74–79.
22. King JN, Maurer MP, Altmann BO, Strehlau GA. Pharmacokinetics of clomipramine in dogs following single-dose and repeated-dose oral administration. Am J Vet Res 2000;61:80–85.
23. Crowell-Davis SL, Seibert LM, Sung W, Parthasarathy V, Curtis TM. Use of clomipramine, alprazolam and behavior modification for treatment of storm phobia in dogs. J Am Vet Med Assoc 2003;222: 744–748.
24. Hewson CJ, Conlon PD, Luescher UA, Ball RO. The pharmacokinetics of clomipramine and desmethylclomipramine in dogs: parameter

estimates following a single oral dose and 28 consecutive daily oral doses of clomipramine. J Vet Pharmacol Ther 1998;21:214–222.
25. Pouchelon JL, Martel E, Champeroux P, Richard S, King JN. Effects of clomipramine hydrochloride on heart rate and rhythm in healthy dogs. Am Vet J Res 2000;61:960–964.
26. SAS Institute. SAS User's guide: Statistics (Version 5), Cary, North Carolina, 1985.
27. Sanders WL, Gaynor PJ. Analysis of switchback data using Statistical Analysis System, Inc., software. J. Dairy Sci 1987;70:2186–2191.
28. Knol BW, Dieleman SJ, Bevers MM, van den Brom WE, Molt JA. Effects of methods used for blood collection on plasma concentrations of luteinising hormone, testosterone and cortisol in male dogs. Vet Q 1992;14:126–129.
29. Feldman EC, Nelson RW. Canine hyperadrenocorticism. In: Canine and Feline Endocrinology and Reproduction, 3rd ed. Philadelphia: Saunders, 2004:252–357.
30. Thun R, Eggenberger E, Zerobin K. 24-hour profiles of plasma cortisol and testosterone in the male dog: Absence of circadian rythmicity, seasonal influence and hormonal interrelationships. Reprod Domest Anim 1990;25:68–77.
31. Kemppainen RJ, Sartin JL. Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol and thyroxine in dogs. J Endocrinol 1984;103:219–226.
32. Neilson JC. Fear of places or things. In: Horwitz D, Mills D, Heath S, eds. BSAVA Manual of Canine and Feline Behavioral Medicine. Gloucester, UK: Br Small Anim Vet Assoc, 2002:173–180.
33. Schwizgebel D. Zusammenhänge zwischen dem Verhalten des Tierlehrers und dem Verhalten des Deutschen Schäferhundes im Hinblick auf tiergerechte Ausbildung. Aktuelle Arbeiten zur artgemassen Tierhaltung, 1982:138–148.
34. Hetts S, Clark JD, Arnold CE, Mateo JM. Influence of housing conditions on beagles behavior. Appl Anim Behav Sci 1992;34:197–155.
35. Overall KL. Clinical Behavioral Medicine for Small Animals. St. Louis: Mosby-Year Book, 1997:261–262.
36. King JN, Simpson BS, Overall KL, et al. Treatment of separation anxiety in dogs with clomipramine: results from a prospective, randomized, double-blind, placebo-controlled, parallel-group, multicenter clinical trial. Appl Anim Behav Sci. 2000;67:255–275.
37. Plumb D. Veterinary Drug Handbook, 4th ed., Ames, Iowa: Iowa State Univ Pr, 2002:1–3.
38. Mills DS, Simpson BS. Psychotropic agents. In: Horwitz D, Mills D, Heath S, eds. BSAVA Manual of Canine and Feline Behavioral Medicine. Gloucester, UK: Br Small Anim Vet Assoc, 2002:237–248.
39. Taylor CB. Treatment of anxiety disorders. In: Schatzberg AF, Nemeroff CB, eds. Essentials of Clinical Psychopharmacology. Washington DC, Am Psychiatric Publ, 2001:431–445.
40. Plumb D. Veterinary Drug Handbook, 4th ed. Ames, Iowa: Iowa State Univ Pr, 2002:148–149.
41. Potter WZ, Manji HK, Rudorfer MV. Tricyclics and tetracyclics. In: Schatzberg AF, Nemeroff CB, eds. Essentials of Clinical Psychopharmacology. Washington DC, Am Psychiatric Publ, 2001: 5–26.
42. Hansen-Grant SM, Pariante CM, Kalin NH, Miller AH. Neuroendocrine and immune system pathology in psychiatric disease. In: Schatzberg AF, Nemeroff CB, eds. Essentials of Clinical Psychopharmacology, Washington DC, Am Psychiatric Publ, 2001:171–194.
43. Seibert LM, Crowell-Davis SL, Wilson GH, Ritchie BW. Placebo-controlled clomipramine trial for the treatment of feather picking disorder in cockatoos. J Am Vet Anim Hosp 2004;40:261–269.
44. Pryor PA, Hart BL, Cliff KD, Bain MJ. Effects of a selective serotonin reuptake inhibitor on urine-spraying behavior in cats. J Am Vet Med Assoc 2001;219:1557–1561.
45. Seksel K, Lindemans MJ. Use of clomipramine in the treatment of anxiety-related and obsessive-compulsive disorders in cats. Aust Vet J 1998;76:317–321.
46. Seksel K, Lindemans MJ. Use of clomipramine in the treatment of obsessive-compulsive disorder, separation anxiety and noise phobia in dogs: a preliminary, clinical study. Aust Vet J 2001;79:252–256.
47. Warriss PD. The welfare of animals during transport. In: Raw M-E, Parkinson J, eds. The Veterinary Annual. Cambridge: Blackwell Sci, 1996;36:73–85.
48. Rushen J. Some problems with the physiological concept of "stress". Aust Vet J 1986;63:359–361.
49. Wolfle TL. Understanding the role of stress in animal welfare: practical considerations. Moberg GP, Mench JA, eds. The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Wallingford, UK, CABI Publ, 2000:351–368.
50. Rushen J. Some issues in the interpretation of behavioral responses to stress. Moberg GP, Mench JA, eds. The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare. Wallingford, UK, CABI Publ, 2000:23–42.
51. Beerda B. Stress and Well-being in Dogs. Utrecht: Univ Utrecht, 1997: 105–119.