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Abstract

Twenty-four beagles were used to measure physiological and behavioral reactions to air transport. Each of 3 groups of 4 sedated (with 0.5 mg/kg body weight of acepromazine maleate) and 4 non-sedated (control) dogs was flown on a separate flight between Montreal, Quebec, and Toronto, Ontario, after being transported by road from Quebec City to Montreal. Saliva and blood samples were taken before ground and air transport and after air transport. The heart rate was monitored during the whole experiment except during ground transport, and behavior was monitored by video during air transport. Sedation did not affect any of the variables measured. The mean plasma cortisol concentration was significantly higher ($P < 0.05$) after ground transport than at baseline (225.3 vs 134.5 nmol/L); the mean salivary cortisol concentration was significantly higher ($P < 0.05$) after both ground and air transport than at baseline (16.2 and 14.8, respectively, vs 12.6 nmol/L). The mean neutrophil count was significantly higher ($P < 0.05$) after both ground and air transport than at baseline (80.6 and 81.4, respectively, vs 69.5 per 100 white blood cells), whereas the mean lymphocyte count was significantly lower ($P < 0.05$) (13.2 and 13.7, respectively, vs 22.4 per 100 white blood cells). Loading and unloading procedures caused the largest increase in heart rate. On average, the dogs spent more than 50% of the time lying down, and they remained inactive for approximately 75% of the time, except during take-off. These results suggest that transportation is stressful for dogs and that sedation with acepromazine, at the dosage and timing used, does not affect the physiological and behavioral stress responses of dogs to air transport.

Résumé

Vingt-quatre chiens de race Beagle furent utilisés pour mesurer les réactions physiologiques et comportementales au transport aérien. Trois groupes de 4 chiens soumis à une sédation (0,5 mg/kg de poids corporel de maléate d'acépromazine) et 4 chiens témoins ne recevant pas de sédatif furent transportés sur des vols séparés entre Montréal, Québec et Toronto, Ontario après avoir été transportés par voie terrestre entre Québec et Montréal. Des échantillons de salive et de sang furent prélevés avant le transport terrestre et aérien et après le transport aérien. Le rythme cardiaque fut surveillé durant la durée de l'expérience sauf durant le transport terrestre, et le comportement fut surveillé par vidéo durant le transport aérien. La sédation n'affecta aucune des variables mesurées. La concentration moyenne du cortisol plasmatique après le transport terrestre était significativement plus élevée ($P < 0,05$) que la valeur de base (225,3 vs 134,5 nmol/L); la concentration moyenne du cortisol dans la salive après le transport terrestre et le transport aérien était significativement plus élevée ($P < 0,05$) que la valeur de base (16,2 et 14,8, respectivement vs 12,6 nmol/L). Le compte moyen des neutrophiles après le transport terrestre et le transport aérien était significativement plus élevé ($P < 0,05$) que la valeur de base (80,6 et 81,4, respectivement vs 69,5 par 100 globules blancs), alors que le compte moyen des lymphocytes était significativement plus faible ($P < 0,05$) (13,2 et 13,7, respectivement vs 22,4 par 100 globules blancs). Les procédures d'embarquement et de débarquement causèrent la plus grande augmentation du rythme cardiaque. En moyenne, les chiens demeurèrent couché plus de 50 % du temps, et demeurèrent inactif pour environ 75 % du temps, sauf au moment du décollage. Les résultats suggèrent que le transport est une activité stressante pour les chiens et que la sédation avec de l'acépromazine, à la dose et au temps utilisés, n'a pas affecté les réponses physiologiques et comportementales des chiens au transport aérien.

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Introduction

Transportation has been shown to be stressful for many domestic animal species, such as cattle, pigs, poultry, and horses (1,2). However, very little scientific research has been done on the effects of transportation in dogs, much less on the effects of air transport. One study, by Leadon and Mullins (3), found that greyhounds transported in the belly hold of a jet freighter showed a higher stress response than animals kept in the main cargo hold. To alleviate stress during air transport, sedatives are sometimes prescribed (4). The International Air Transport Association's *Live Animal Regulations* (5) warn against tranquilizing pets prior to transport. Tennyson (6) reported that, at a meeting of the United States Department of Agriculture and airline officials, it was suggested that "... oversedation is the most frequent cause of animal deaths during airline transport. . . . Investigations revealed that almost half the deaths resulted from sedation. The second most frequent cause of death was environmental stress, especially in brachycephalic breeds. Third in frequency were disease complications from coronavirus, parvovirus, and respiratory diseases that were not evident during examination, but had a sudden debilitating onset with the stress of transport at high altitude. Least common, in fact, rare, were deaths caused by mishandling by the carriers."

Since existing regulations and recommendations regarding transportation of dogs do not appear to be founded on scientific data, we conducted a study to determine to what extent air transport is stressful for dogs. Our second objective was to compare physiological and behavioral reactions of sedated and non-sedated dogs during air transport.

Materials and methods

Animals and treatments

Twenty-four beagles, 13 males and 11 females, with an average weight of 15 kg and an average age of 14 mo, were used. The experiment was conducted according to the Canadian Council on Animal Care guidelines (7). The dogs had been reared at the Laval University, Quebec, animal care facility, were in good health, and had not previously traveled by air. Even though they had been socialized and were used to being handled, they were laboratory dogs and did not behave like typical pets. They were divided into 3 groups that were flown on 3 separate flights (January 4, 11, and 13, 2000). Each group included 4 control dogs (total: 6 males and 6 females) and 4 sedated dogs (total: 7 males and 5 females).

The sedative used, acepromazine maleate (Atravet; Wyeth-Ayerst Laboratories, Guelph, Ontario), was given orally at the minimum dose recommended by the manufacturer, 0.5 mg/kg body weight (BW), at least 30 min before the pre-air-transport blood sampling. The minimum dose was used in order to avoid adverse reactions. All departures were delayed because of bad weather and, as a result, the sedative was administered on average 5 h before take-off (3.5, 6, and 6 h, respectively, for flights 1, 2, and 3).

Experimental phases and data collection

On the day prior to transportation, the dogs were shaved to expose the skin overlying the jugular vein, in order to facilitate blood

sampling. A patch was also shaved lateral to the sternum, in order to facilitate heart rate monitoring. On the morning of transportation, after food had been withheld overnight, the dogs were prepared for departure. Each dog was taken to an examination table, and a baseline blood sample was quickly drawn from the jugular vein with a 5-mL EDTA Vacutainer tube (Becton Dickinson, Franklin Lakes, New Jersey, USA) and a 20-gauge needle. A baseline saliva sample was taken with a cotton swab wrapped around a wood wand. The swab was then sealed in a 13-mm plastic tube. To stimulate salivation, a small quantity of citric acid crystals (Laboratoire Valmo, Chambly, Quebec) was put on the dog's tongue prior to sampling (8). Before being placed in a plastic transportation kennel (Skykennel; Dorskocil Manufacturing Company, Arlington, Texas, USA), each dog was weighed and its baseline rectal temperature measured.

The dogs were transported by road in a minivan to the Dorval Airport (about 300 km from the animal care facility). Upon arrival at the airport, the dogs were given the sedative and transferred to a cargo hangar, where saliva and blood samples were taken again (pretransport samples). Blood smears were prepared, and tubes were placed on ice. The dogs were then fitted with a heart rate monitor (Polar XTrainer Plus; Polar, Richard Browne & Co., North York, Ontario), which stored data at 15-s intervals; its transmitting belt was covered with an elastic bandage (Vetrap; 3M Company, Montreal, Quebec), to keep it in place. A cotton jacket was put on the dog to protect the monitor, and the animal was returned to its kennel. A battery-powered camera (BCD 468; Vidamax Canada, Laval, Quebec) and an autonomous lighting system that shone light inside the kennel were mounted on the outside of each kennel for recording behavior. Cameras were connected to 4 input sequential switchers (SDR4; Vidamax Canada). Each kennel was fitted with sensors (Onset Computer, Pocasset, Massachusetts, USA) for air temperature (StowAway XTI02 and Hobo Temp, H8-002-02) and relative humidity (StowAway RH, SRHA02). The accuracy of the temperature sensors was $\pm 0.25^{\circ}\text{C}$. For the humidity sensors, an accuracy of 3% to 5% is reported for the range measured. An additional Hobo pressure sensor (HPA-0015) was used to monitor air pressure variations during flight. Measurements were made at 60-s intervals.

At the cargo hangar, the kennels were reloaded into the minivan, transported to the loading ramp of the aircraft, and then loaded with a conveyer belt into the belly cargo hold of a Boeing 757-200 aircraft. The kennels were secured in the cargo space with belts and binders, and 2 battery-operated video recorders (AG-1070; Panasonic, Secaucus, New Jersey, USA) and the sequential switchers were turned on. The video-recording system allowed for the use of a scan sampling technique, by which each dog of a group of 4 was filmed for 15 s. The dogs were then flown to Pearson International Airport, Toronto (average flying time, 48 min).

Upon arrival, the aircraft was unloaded and reloaded, then flown back to Dorval (average flying time, 43 min). The kennels remained in the cargo hold during turn-around (70 min on average). After arrival at Dorval, the kennels were unloaded and transported back by minivan to the cargo hangar for measurement of rectal temperature and sampling of blood and saliva (post transport samples). The heart rate monitoring systems and sensors were retrieved at that time. Blood smears were prepared, and the dogs

Table I. Plasma and salivary cortisol concentrations and white blood cell (WBC) counts and ratio [mean (and standard error)] before and after air transport in non-sedated [control (C)] and sedated (S) dogs

Variable	Before ground transport (baseline)		Before air transport		After air transport	
	C	S	C	S	C	S
Cortisol concentration (nmol/L)						
Plasma	146.2 (24.6)	122.8 (22.3)	215.4 ^a (32.4)	235.2 ^a (49.0)	115.0 (26.8)	191.5 (27.4)
Saliva	12.3 (1.2)	12.8 (1.2)	16.8 ^a (2.0)	15.6 ^a (2.1)	15.3 ^a (2.5)	14.4 ^a (1.8)
Cell count (/100 WBCs)						
Neutrophils	71.2 (2.6)	67.9 (1.7)	78.2 ^a (2.0)	83.0 ^a (1.4)	82.8 ^a (2.1)	80.0 ^a (2.8)
Lymphocytes	20.3 (2.1)	24.6 (1.6)	15.3 ^a (1.8)	11.2 ^a (1.3)	11.6 ^a (2.0)	15.8 ^a (2.6)
Neutrophil:lymphocyte ratio	4.3 (0.9)	2.9 (0.3)	5.8 ^a (0.6)	9.1 ^a (1.4)	10.0 ^a (1.9)	7.2 ^a (1.6)

^a Significantly different from baseline value ($P < 0.05$)

were given water and allowed to walk around before returning to the animal care facility by road.

The blood samples were spun in a refrigerated centrifuge at Laval University within 7 h of the dogs' arrival at Dorval, and plasma was transferred to 2-mL micro tubes (Sarstedt, Nümbrecht, Germany). The saliva and plasma samples were kept frozen at -18°C until analysis. After air drying, slides were fixed for 30 s in anhydrous methanol and later stained with Wright's-Giemsa stain for 43 min to allow for the differential white blood cell (WBC) count (100 cells).

Salivary and plasma cortisol concentrations were measured with a radioimmunoassay method involving a double antibody technique (goat anti-rabbit IgG and rabbit anti-cortisol serum, coated to a 96-well plate of high binding capacity) and iodine-125-labeled cortisol-3-iodohistamine prepared in-house. A liquid scintillation plate counter was used to determine end-point bound activity. The sensitivity of the method, derived from a precision profile, was calculated to be 5 pg/100 μL (137.9 pmol/L). Coefficients of variation ranged from 6% to 13% over concentrations ranging from 12.5 to 800 pg/100 μL (0.34 to 22.07 nmol/L). Regression analysis of actual vs calculated values, covering a dilution range from 1:2 to 1:240, demonstrated excellent linearity ($r = 0.9972$). The assay requires dilution of plasma, or serum, by approximately 1:100. However, the exact dilution depends on the concentration of cortisol in the sample. Thus, the assay is sufficiently flexible to permit cortisol determination in samples of saliva or plasma from a variety of species.

The videotapes were viewed and behavior was sampled at 1-min intervals. The posture (lying, sitting, or standing) and the activity of each dog (sniffing, remaining immobile, sleeping, looking around) were observed continuously for 15 s. The proportion of time spent in each posture and behavior was then calculated separately for the following periods: waiting at Dorval (30-min period before take-off), take-off from Dorval (10-min period before pressure change), flight from Dorval to Toronto, landing in Toronto (10-min period, starting 2 min before pressure change), and turn-around in Toronto.

The heart rate was averaged by 5-min periods for the duration of sampling. The average rate was also calculated for loading at Dorval (30-min period commencing with departure from hangar),

waiting at Dorval, take-off from Dorval, flight from Dorval to Toronto, landing in Toronto, and turn-around in Toronto, as well as for each period of the return trip to Dorval. The maximum heart rate was also determined for the same periods.

Statistical analysis

The general linear model of SAS (9) was used to analyze the cortisol concentration, WBC count, heart rate, and behavioral data, using the repeated measures analysis. To determine the effects of sedation on cortisol and WBC differential-count variables, the baseline values (before sedation) were excluded from the first analysis. Transport effects on the same variables were determined from the entire data set with a second analysis, in which multiple comparisons were made between the baseline samples (collected at Laval University) and the pre-air-transport samples (collected at Dorval Airport), as well as between the baseline samples and the post-transport samples (collected at Dorval Airport). Multiple comparisons were done between loading at Dorval and each subsequent period for heart rate and between waiting and each subsequent period for behavioral data. Differences were considered significant when the P value was less than 0.05.

Results

The experiment was conducted in January, under cold, humid, and windy conditions. The temperature in the cargo hold while the kennels were on board the aircraft and the cargo hold door was closed ranged from 15.7 to 18.8, 14.3 to 17.6, and 7.0 to 15.4 $^{\circ}\text{C}$, for flights 1, 2, and 3, respectively. Relative humidity ranged from 25.7% to 42.4%, 23.1% to 42.8%, and 9.8% to 23.5%, respectively, for flights 1, 2, and 3.

Plasma and salivary cortisol concentrations

The plasma cortisol concentration (Table I) was not significantly affected by sedation, although it tended ($P = 0.06$) to be higher in the sedated dogs than in the control dogs after air transport. The pre-air-transport value was significantly higher than the baseline value. The

Table II. Maximum heart rate (beats/min; mean \pm standard deviation) recorded for non-sedated and sedated dogs at each experimental period (flights 2 and 3)

Experimental period	Non-sedated (n = 3)	Sedated (n = 3)
Loading	187 \pm 5	176 \pm 29
Waiting	153 \pm 49	168 \pm 30
Take-off	151 \pm 29	160 \pm 42
Flying	162 \pm 31	162 \pm 32
Landing	108 \pm 52	79 \pm 18
Turn-around	164 \pm 41	176 \pm 18
Take-off	150 \pm 50	152 \pm 20
Flying	124 \pm 38	160 \pm 61
Landing	148 \pm 68	99 \pm 29
Unloading	181 \pm 32	187 \pm 14

post-air-transport value was also higher than the baseline value in the sedated animals, but the difference was not significant. On the other hand, the salivary cortisol concentration (Table I) was significantly higher before and after air transport than at baseline; it was not affected by sedation.

WBC counts

Sedation did not affect any of the variables from the WBC count (Table I). The neutrophil count was significantly higher before and after air transport compared with baseline. Opposite results were found for the lymphocyte count. Consequently, the neutrophil:lymphocyte ratio was significantly higher in the pre- and post-transport periods compared with baseline.

Heart rate

Because of technical problems, there was a full data set during the 1st flight for only 1 dog. This flight was not considered in the analysis. Sedation did not significantly affect heart rate. During most of the measurement periods, the average heart rate remained in the range of 80 to 90 beats/min, although peak values of over 180 beats/min were recorded for some of these periods (Table II). Loading and unloading procedures caused the largest increase in heart rate. Heart rate was significantly higher during loading than during take-off, flight, landing, turn-around, or any subsequent period except waiting and unloading (Figure 1).

Behavior

Behavior was highly variable among the animals and was not significantly affected by sedation or the experimental period (Figure 2). On average, the dogs spent more than 50% of the time lying down and were sitting the rest of the time. Very little standing was observed. The dogs remained inactive more than 75% of the time during waiting, flying, landing, and turn-around. During take-off, non-sedated dogs appeared to be more active than sedated dogs, but the difference was not statistically significant, owing to the high variability in the dogs' response. There was no evidence of excitement, such as digging, scraping, or barking (3). Also, behaviors such as mouth and snout licking, paw lifting, and body shaking, which are thought to indicate acute stress (10), were not

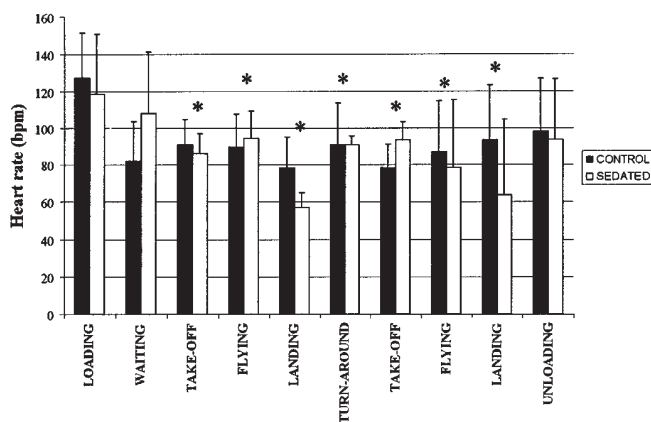


Figure 1. Heart rate, beats/min (bpm) [mean and standard deviation (SD)], of sedated and non-sedated (control) dogs during air transport between Dorval, Quebec, and Toronto, Ontario. *Significantly different from rate during loading period ($P < 0.05$).

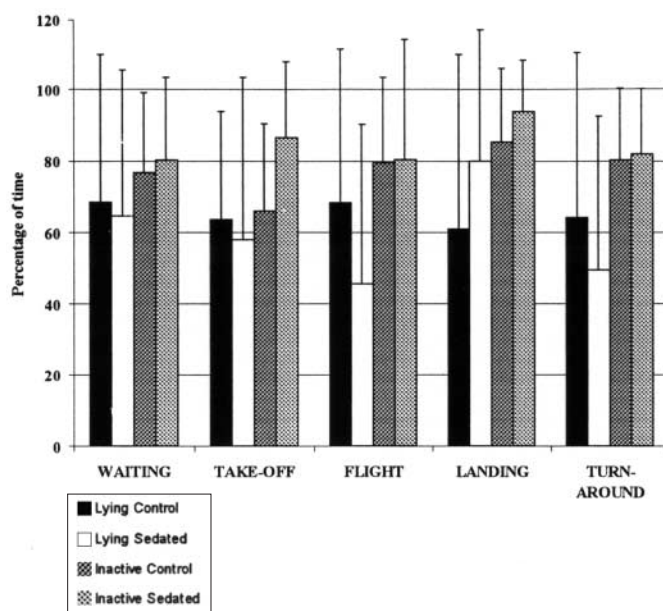


Figure 2. Percentage of time (mean and SD) spent lying down and inactive by sedated and non-sedated dogs during air transport.

recorded at a frequency worth reporting. However, because of the time sampling technique used, many occurrences of these events with a very short duration may have been missed.

Rectal temperature

Rectal temperatures taken at the animal care facility, as well as before and after the flights, did not differ significantly between the groups or between the sampling times. The average value (± 1 standard deviation) was $38.5 \pm 0.7^\circ\text{C}$.

Discussion

The longer-than-expected delays between acepromazine administration and take-off in our experiment likely explain the lack of effect of sedation on the physiological and behavioral variables

measured during the flight. A pharmacokinetic study showed that the sedative effect of an oral dose of 1.3 to 1.5 mg/kg BW lasts about 4 h (11). In our experiment, the drug was administered at a dose of 0.5 mg/kg BW 5 h before take-off, on average. However, heart rate was also measured before the flight left Dorval, at a time when the medication should still have been fully effective, and no significant differences were found between sedated and non-sedated dogs; in fact, a great deal of variability between individual animals was observed. Zachary (4) reported that individual dogs may react differently to sedation, which could explain the lack of consistent effects of acepromazine.

Despite a lack of effect of sedation, physiological variables clearly indicated that transportation, whether by road or air, was stressful for the dogs in this experiment. The average baseline salivary cortisol concentration, measured at the animal care facility (12.6 nmol/L), was higher than the average basal level of 6.0 nmol/L measured by Beerda et al (8) in adult dogs of various breeds and the average value of 5.3 nmol/L reported by Vincent and Michell (12) for adult beagles. This suggests that our dogs may already have been excited at the time of initial sampling, probably owing to the handling by human beings. They showed signs of excitement when they saw humans enter their enclosure. The average salivary cortisol concentrations before (16.2 nmol/L) and after (14.8 nmol/L) air transport were significantly higher than the baseline levels, suggesting stress due to transport. However, they were lower than the maximum values measured by Beerda et al (8) in dogs submitted to several stressful stimuli, such as a loud noise (20.4 nmol/L), a falling bag (18.7 nmol/L), and an electric shock (15.5 nmol/L). These discrepancies are probably due to differences in the timing of sampling following the onset of the stressor and to the duration of the stressor. The dogs in our study may have shown some habituation to the transport stressor by the time sampling was performed, whereas in the experiment of Beerda et al the samples were taken shortly after the onset of the acute stimuli; the cortisol levels in the latter investigation reached a maximum between 16 and 20 min after the stressful event and decreased thereafter. In contrast to other species, no circadian rhythm in cortisol levels has been detected in dogs (13). Therefore, the increases observed in our experiment are likely to be due to transport.

The average baseline cortisol values in plasma (134.5 nmol/L, or 4.9 µg/dL) and saliva (12.6 nmol/L, or 0.46 µg/dL) in the dogs we studied were higher than the average (plasma: 86.2 nmol/L; saliva: 5.3 nmol/L) and maximal baseline values (plasma: 125 nmol/L; saliva: 10 nmol/L) in the adult beagles studied by Vincent and Michell (12). The plasma levels measured after road and air transport in our experiment were, on average, higher (189.2 nmol/L, or 6.9 µg/dL) than the baseline value, which suggests stress due to transport. In fact, these values compare well to the ones reported by Leadon and Mullins (3) for greyhound dogs before and after air transport in the main cargo hold (7.1 to 8.6 µg/dL). The tendency for a lower plasma cortisol level after air transport for control dogs compared with sedated dogs is difficult to explain and was not confirmed by the salivary cortisol values, which remained higher after air transport compared with baseline (12.6 nmol/L) for both control (15.3 nmol/L) and sedated (14.4 nmol/L) dogs.

Jasper and Jain (14) showed that injection of corticosteroids or adrenocorticotrophic hormone in dogs causes an increase in neutrophils and a reduction in lymphocytes, the changes beginning 2 to 4 h after injection. High levels of cortisol fed to pigs have been shown to increase their neutrophil:lymphocyte ratio, by increasing the number of neutrophils and decreasing the number of lymphocytes (15). A similar response to an increase in cortisol was observed in our experiment: the ratio was indeed higher in the pre- and post-transport periods compared with baseline, suggesting, once again, that the dogs were stressed by both road and air transport. Leukocyte counts are subject to diurnal variations: the neutrophil count increases during the day and reaches its maximum value around 19:00, whereas the lymphocyte count reaches its maximum value around 23:00, the percentage increase between the minimum value (at 7:00) and the maximum value being 50% (16). Since the highest lymphocyte count in our experiment was obtained in the morning, prior to leaving Laval University, and the lowest count was obtained in the afternoon and late evening, at Dorval Airport, it is likely that the differences observed were due to stress and the resulting cortisol secretion and not simply to normal diurnal variation.

The heart rate observations suggest that the most stressful steps of air transport are loading and unloading. Similar observations have been made in farm animals. According to Tennesen, Price, and Berg (17), loading of cattle onto a truck is accompanied by a large increase in heart rate, which is partly due to the novelty of the process. The change in the environment and the handling associated with loading and unloading in our experiment were likely responsible for the increased heart rate in the dogs. Even though high peaks in heart rate (over 180 beats/min) were observed for each dog during the other observation periods, the rate remained, on average, within a more normal range. Beerda et al (8) reported a mean undisturbed heart rate of 75 beats/min for adult dogs of various breeds; when the dogs were exposed to acute stimuli, their mean heart rate ranged from 100 to 130 beats/min during the first few minutes of the stress response, peak values being 146 to 181 beats/min.

It seems that the dogs in our experiment were submitted to acute stressors during each step of transport but that their stress response was not sustained. They were mainly inactive during each observation period, and no differences were observed between observation periods. If we consider that behavioral recordings were not made during the loading period, this is fairly consistent with the heart rate observations. However, it should not be concluded that the dogs were not stressed simply because they remained inactive for most of the trip. In fact, Beerda et al (10) hypothesized that beagles may tend to adopt a "conservative-withdrawal" response, characterized by passive behavior, rather than a more active "fight-or-flight" response, when confronted with a stressful situation.

Our results suggest that both road and air transport are stressful for dogs, at least for animals who are not used to travel. Sedation with acepromazine, at the dosage used, did not significantly affect the stress reaction during air transport. This may be explained by the longer-than-expected delays between administration of the drug and take-off of each flight. It may also have been due to a large individual variation in the dogs' response to the sedative.

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