Plasma Cortisol Levels of Dogs at a County Animal Shelter

MICHAEL B. HENNESSY,1 HARRY N. DAVIS, MICHAEL T. WILLIAMS, CAROLYN MELLOTT AND CHET W. DOUGLAS

Department of Psychology, Wright State University, Dayton, OH 45435 USA

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HENNESSY, M. B., H. N. DAVIS, M. T. WILLIAMS, C. MELLOTT AND C. W. DOUGLAS. Plasma cortisol levels of dogs in a county animal shelter. PHYSIOL BEHAV 62(3) 485–490, 1997.—Plasma cortisol levels were examined to assess the stress of dogs in a county animal shelter. Groups of dogs confined in the shelter for their 1st, 2nd, or 3rd day had higher cortisol levels than dogs that had been there for more than 9 days. Dogs in the shelter for an intermediate period (Day 4–9) had intermediate levels of cortisol. The cortisol concentrations of dogs during their first day in the shelter were greater than either those of the same dogs on Day 4/5 in the shelter or those of a group of pet dogs sampled in their own homes. There was no overall effect of 20 min of social interaction with a human (e.g., petting) on the plasma cortisol levels of dogs in the shelter on Day 1–3. However, the gender of the petter did affect cortisol levels. Those dogs interacting with a female had lower cortisol concentrations at the end of the session than dogs interacting with a male. The results suggest that confinement in a public animal shelter produces a prolonged activation of the hypothalamic-pituitary-adrenal axis. Further, it appears that some subtle aspect of interaction with a human may be capable of moderating this response. Possible implications for the welfare of confined dogs, and for the development of behavior problems in dogs obtained from shelters, are discussed. © 1997 Elsevier Science Inc.

PSYCHOGENIC stressors have long been recognized as potent stimuli for hypothalamic-pituitary-adrenal (HPA) activation (14,15). Among the most effective of such stimuli are exposure to novel surroundings and separation from social attachment figures. Studies with laboratory animals, particularly rodents and primates, have shown that isolation in a novel environment is a reliable and powerful elicitor of HPA activation (7,12,17). Rats simply placed alone into an unfamiliar apparatus frequently exhibit elevations of plasma adrenocorticoïd levels that are as great as those seen in animals receiving electric shock in the apparatus (1,2,7). Exposure to a novel environment together with a familiar social partner often results in increased HPA activity (10,13). However, the presence of a specific attachment figure (e.g., an infant rhesus’ or squirrel monkey’s mother) can reduce the adrenocorticoïd response in a novel environment, and brief separation from an attachment figure can produce a vigorous adrenocorticoïd response even if the separation occurs in familiar surroundings (11,23,26).

Recently, we reported that domestic dogs placed alone into a modestly novel environment for 4 h exhibited an elevation of plasma adrenocorticoïd levels (24). Interestingly, the presence of the dog’s human caretaker sitting quietly in the room significantly reduced this response, but the presence of the dog’s long-standing kennel mate did not. The most extreme form of social isolation in novel surroundings that most domestic dogs are likely to encounter is impoundment in a public animal shelter. While separated from human and any previous canine companions, the impounded dog is assailed with novelty, not only in terms of its physical surroundings, but also by constant exposure to novel animals, unfamiliar noise, and the disruption of familiar routines (e.g., feeding schedules, walks for elimination).

To our knowledge, the potential impact of this complex “naturalistic” stressor on the HPA activity of domestic dogs has yet to be explored. Moreover, examination of this issue might have practical significance, both for the welfare of confined dogs, and possibly for better understanding the behavior of adopted dogs. It has been suggested that the trauma associated with abandonment may lead to some of the behavior problems which are commonly observed in dogs adopted from shelters (25). In the current study, we examined the plasma cortisol levels of dogs held in a county animal shelter for different numbers of days, compared the cortisol levels of sheltered dogs to those of dogs maintained as house pets, and attempted to moderate cortisol elevations in sheltered dogs through direct human contact.

GENERAL METHOD

Subjects and Housing

The subjects were dogs of various and mixed breeds. With the exception of one group discussed under Experiment 1, the...
animals were housed in the Montgomery County Animal Shelter, a large, progressive metropolitan shelter serving Dayton, Ohio. As is typical of most such shelters, the population included strays, dogs abandoned or brought in by their owners for various reasons, and dogs seized by shelter staff because of neglect or other violations. It was not possible to clearly document the source of many dogs, so no attempt was made to distinguish subjects on the basis of provenance.

The portion of the shelter used for housing dogs was divided into four areas: three general housing rooms designated “Yellow”, “Green”, and “Red”, and a smaller “Adoption Room” used to display dogs for adoption. (After the facility closed to the public for the day, dogs in the Adoption Room were brought to the Yellow Room for housing over night.) The rooms were illuminated with a combination of overhead fluorescent and natural lighting. The fluorescent lamps were generally illuminated 24 h/day. In all rooms, dogs were almost always housed individually with a rare exception in which two littermates or other dogs taken from the same location were housed together. Most dogs were chosen for our work from the Yellow and Green Rooms, which were large rooms (11.4 × 5.5 m) with banks of 30 metal cages of different sizes (0.6–1.2 × 0.7 × 0.6–0.7 m) along each of the long walls, and a row of six free-standing pens (0.9–1.9 × 1.5 × 1.5 m) in the middle of the open area. On test days, the number of dogs in each of these rooms varied greatly, from a few to nearly full capacity. The rooms were noisy due to the clanging of metal cages and bowls, the playing of a radio over the intercom, and the barking of dogs in the same and neighboring rooms. No subjects were drawn from the Red Room, which was reserved for dogs that were sick, aggressive, or otherwise unsuitable for placement into homes. Dogs from the Adoption Room were in Experiment 1. This room was smaller (5.5 × 3.8 m) and less noisy than the other rooms. Dogs in the Adoption Room had temperaments assessed to be especially suitable for house pets (e.g., not overly excitable).

All dogs arriving at the shelter were, by law, kept a minimum of 3 days unless they either were claimed by their owners or were too injured or sick to keep. Dogs in the Yellow and Green Rooms were often kept for additional days. Those healthy dogs not claimed were kept an average of six days before it was decided whether they would be placed up for adoption or destroyed. Dogs placed into the Adoption Room were generally kept until they were adopted.

Subjects were selected from among all those apparently healthy, nonlactating, postweaning dogs that did not appear likely to bite, as judged by body posture, timidity, and breed (i.e., we did not choose dogs that barked aggressively at the front of the cage and trembled at our approach, or were of reputed aggressive breeds, notably, rottweilers, pitbulls and chows). It should be pointed out, however, that the clear majority of dogs in the Yellow, Green, and Adoption Rooms were highly socialized towards humans and actively solicited social contact by such behaviors as tail-wagging, yelping, and attempting to lick passing humans through the mesh of the cage. In all, our sample seemed to be quite representative of the population of dogs that might be considered for adoption at many public shelters.

Prior to each dog’s test, we recorded its gender, the room from which it was taken, and its date of arrival in the shelter. We also recorded rough estimates of the dog’s weight (small: <20 lb; medium: 20–50 lb; large: 50–100 lb; or, extra large: >100 lb) and age (young: <1 yr; adult: 1–10 yr; old: >10 yr). Estimates of age were aided by inspection of dentition (presence/absence of puppy teeth, spacing and wear of teeth). Although we recorded gender, it should be noted that groups were a heterogeneous assortment of prepubertal and adult, intact and gonadectomized dogs.

Blood-Sample Collection and Hormone Determination

Blood samples (~0.5 ml) were collected from the cephalic vein with a heparinized injection syringe. A “holder” or two gently restrained the dog and spoke soothingly to it while extending one of the dog’s front legs to another individual who performed the venipuncture. In the shelter, dogs were taken to a nearby procedure room for sample collection. Across both experiments, 88% of these blood samples were collected within 4 min of the onset of disturbance (e.g., opening of the animal’s cage door). Based on data from rodents, this was rapidly enough to ensure that the cortisol levels in the samples collected were not appreciably affected by the sampling procedure (4,6,19). However, even for the remaining 12% of samples, which took between 4 and 6 min to collect, there was no indication that cortisol levels were elevated by the sampling procedure (i.e., the majority of these dogs had cortisol concentrations below their group mean). This is not entirely surprising since the initial portion of the dog’s experience with sampling (e.g., being taken from cage, receipt of attention from humans) was probably more pleasurable than stressful.

Samples were stored on ice until centrifugation to separate plasma, which was then frozen until assayed. Duplicate aliquots were assayed for cortisol using a 125I radioimmunoassay kit (“Coat-a-Count”, Diagnostic Products Corporation, Los Angeles, CA). The dog adrenal secretes significant quantities of both cortisol and corticosterone, but we previously found that plasma levels of these two hormones showed similar patterns of fluctuations during separation and exposure to novelty (24). Intra- and interassay coefficients of variation were each less than 6%.

EXPERIMENT 1

This experiment used both cross-sectional and longitudinal designs to assess the plasma cortisol levels of dogs housed in the shelter for differing numbers of days. An additional comparison was made between the cortisol levels of a group of dogs during their first day in the shelter and those of a group of dogs kept as house pets and sampled at their own homes.

Method

For the cross-sectional portion of the experiment, there were seven groups of dogs (approximately equal numbers of males and females in each group) defined by the length of the dog’s stay in the shelter. Twenty four dogs had blood samples collected on the day of arrival at the shelter (Day 1), 22 on Day 2, 23 on Day 3, 21 on Day 4, 19 on Day 5, 24 on Day 6–9, and 13 beyond Day 9 (Day 10+; the longest stay in the shelter for dogs in this group was 38 days). For the longitudinal portion of the experiment, additional dogs had blood samples collected on Day 1 and again on Day 4/5, if the dogs were still in the shelter at that time. Fifteen dogs had both samples collected and were included in the longitudinal portion. All samples were collected before the evening feed, between 1600 and 2000 h.

Pet dogs were those of the experimenters, their associates, and others interested in the project. These dogs were sampled at their own homes after the completion of cross-sectional and longitudinal portions of the experiment. Sampling times, which were measured from the dogs’ detecting the approach of experimenters, were comparable to those in the shelter. The comparison group consisted of dogs originally intended to be included in the longitudinal portion of Experiment 1. However, when the Day
Results and Discussion

For the cross-sectional portion of the experiment, a preliminary analysis revealed no effect of gender on cortisol levels. For this reason and because gender in these experiments represents a heterogeneous variable, comprising gonadectomized and intact adults as well as prepubertal animals, it was not included as a factor in subsequent analyses in this or the following experiment.

Visual inspection of cortisol levels in the cross-sectional portion (Fig. 1) indicates a step-wise pattern, with lower levels in those dogs housed longer in the shelter. A one-way analysis of variance (ANOVA) was significant, $F(6,139) = 2.59, p < 0.03$. Post hoc paired comparisons with Duncan’s procedure revealed that cortisol concentrations of dogs in the shelter for their first, second, or third day were greater than those of dogs in the shelter for 10+ days ($p < 0.05$). To assess possible influences of age and weight, we next pooled data from dogs in the cross-sectional portion of the experiment that were sampled on Days 1–3, when cortisol levels were uniformly high. Dogs were divided on the basis of estimated age and weight (35 young, 33 adult, 1 old; 32 small, 28 medium, 9 large). Subsequent analyses ($F$ and $t$-tests of various groupings of these classifications) revealed no effect of estimated age or weight on cortisol concentrations.

In the longitudinal portion of the experiment, a pattern of decline in cortisol levels across days was observed. A $t$-test for dependent observations revealed that the drop from Day 1 ($\bar{x} = 20.8 \pm 3.5$ ng/ml) to Day 4/5 ($\bar{x} = 12.7 \pm 1.2$ ng/ml) was significant, $t(14) = 2.60, p < 0.03$. Dogs sampled at their homes were found to have much lower levels of plasma cortisol than did the comparison group of dogs sampled in the shelter on Day 1 [dogs at home, $\bar{x} = 9.6 \pm 1.2$ ng/ml; dogs in shelter, $\bar{x} = 26.5 \pm 4.7$ ng/ml; $t(33) = 3.37, p < 0.01$]. Finally, as expected given the varied recent experience of dogs brought to the shelter, there was considerable variability in cortisol levels on Days 1–3 (see standard errors).

In Experiment 1, we found that the plasma cortisol concentrations of dogs in a county animal shelter for their first, second, or third day were higher than those of dogs in the shelter for a longer period of time. The longitudinal portion of the experiment indicates that this difference was not due solely to a difference in the populations of dogs maintained for short and long periods of time in the shelter. Rather, there was an adaptation of cortisol levels across days.

One difficulty inherent in working in a county animal shelter is that there is no true “nonstressed” group of animals available to provide an estimate of resting or basal cortisol levels. Therefore, there is no standard with which to assess the magnitude of the stress response of impounded dogs. This is particularly problematic because dogs have considerably lower plasma adrenocorticoid levels than do other commonly used laboratory animals. Indeed, the cortisol levels of dogs during stress are no greater than resting levels in other species (5,12,18). In the absence of a basal control group in the shelter, we addressed the issue with what appeared to be the best available strategy: the cortisol levels of dogs in the shelter were compared to those of dogs maintained as house pets. This comparison suggests that the cortisol levels of dogs during their first three days in the shelter represent robust elevations over nonstressed basal levels.

In our previous study, dogs placed into a novel environment for 4 h had lower plasma adrenocorticoid levels when in the presence of their human caretaker sitting quietly than when alone (24). Experiment 2 was designed to investigate whether a somewhat similar manipulation would reduce the cortisol levels of dogs in the shelter. This treatment was of interest primarily because of its potential relevance for the welfare of dogs confined for animal control, experimentation, or other reasons. Unlike our earlier (24) study, the period of interaction was limited to 20 min since interventions of much greater duration would be of little practical value in dog shelters or most other applied settings. To attempt to maximize effectiveness, the human interacted with the dog in a more active fashion than in the Tuber et al. (24) study, and the manipulation was supplemented with the presentation of a food treat.

Method

Based on the results of Experiment 1, dogs were tested during their first three days in the shelter. On each test day, the experimenters first inspected dogs housed in the Yellow and Green Rooms to identify pairs that were of the same gender and had been brought to the shelter on the same day. One dog of each pair was then randomly assigned to a Control Condition and the other to an Experimental Condition. In the Control Condition, the dog was taken to the procedure room where it had a blood sample collected. It was then returned to its cage for 20 min, at which time it was brought back to the procedure room where another blood sample was taken. In the Experimental Condition, the dog was treated in the same fashion except that it remained in the procedure room where a human sat on the floor petting the dog and spoke soothingly to it. The petter had been instructed to gently stroke the dog continuously for the 20 min session. Every 5 min, he/she gave the dog a quarter of a hot dog. The designated petter was never the individual who took blood samples from the dog.

In all, 16 pairs of dogs, eight male and eight female, were included in the experiment. Two male and two female petters were used. Within each condition (Control, Experimental), half of the dogs of each gender were petted by a male, and the other half by a female. Testing in this experiment was performed in the morning between 0900 and 1200 h.
Results and Discussion

Because of heterogeneity of variance, the data from this experiment were subjected to square root transformation prior to analyses. However, the same patterns of significant and marginally significant ANOVA effects were observed for raw and transformed data. For ease of presentation, raw data are shown in the figures. A 2 (Condition) x 2 (Sample) ANOVA, with the last factor treated as a repeated measure, yielded only two main effects that approached significance: Condition, F(1,30) = 3.59, p = 0.068; Sample, F(1,30) = 4.09, p = 0.052. As seen in Fig. 2, the first tendency was for control dogs to have higher cortisol levels than experimental dogs throughout the experiment, despite random assignment. The second tendency was for cortisol levels to be higher following the second sample than following the first, as would be expected if taking the first blood sample elevated cortisol levels 20 min later. There was no indication that the intervention by the petter had any effect on cortisol levels.

However, further visual inspection of data for dogs in the Experimental Condition suggested that the gender of the petter may have had an influence on cortisol levels: of the 8 dogs petted by males, 6 (3 males and 3 females) showed an increase in cortisol levels from the first to the second sample, whereas of the 8 dogs petted by females, 6 (3 males and 3 females) showed a decrease in cortisol levels from the first to the second sample. A 2 (Gender of Petter) x 2 (Sample) ANOVA was then performed on data from dogs in the Experimental Condition (i.e., dogs that were petted). The interaction of the two variables was significant, F(1,14) = 4.67, p < 0.05, Fig. 3. Tests for simple main effects showed that the plasma cortisol levels of dogs petted by males rose from the first to the second sample (p < 0.01), whereas there was no significant change across samples for those dogs petted by females. As a result, those dogs petted by females had lower plasma cortisol levels than did those dogs petted by males following the intervention (p < 0.01), but not prior to it. Thus, the change in cortisol levels with human interaction depended on whether the interaction was with a male or a female. If petted by a male, there was an elevation in cortisol levels, due probably to initial blood-sampling, or possibly to the social encounter itself. Interaction with a female petter prevented such an
increase from occurring. We had originally included the gender of the petter as a variable because of anecdotal reports of those working in the shelter that the dogs often respond more positively to females than to males. The petters were aware of these reports. Yet, our informal impression of the behavior of the dogs during the experiment was that they were calmed by both males and females.

**GENERAL DISCUSSION**

A dog confined in a public animal shelter experiences a whole array of psychogenic stressors, including not only social separation and exposure to novel surroundings, but also noise, restraint, alteration of light-dark cycles and probably of circadian rhythms, disruption of familiar habits, and more generally, unpredictability and loss of control. As expected, dogs housed under these conditions showed elevated cortisol levels. Our data indicate that this response subsides over days, but what perhaps is more noteworthy than the eventual decline in levels is the duration of the response, i.e., that uniformly high levels were observed on Days 1–3.

One must consider that if the general population of dogs arriving at the shelter already had higher cortisol levels than did either those dogs selected to remain in the shelter for 10+ days (largely dogs judged by shelter staff to be suitable for adoption) or actual pets, this population difference could account for differences among groups in the cross-sectional portion of Experiment 1 and in the comparison of shelter dogs to house pets in the same experiment. It was to address such concerns that the longitudinal portion of Experiment 1 was conducted. Because the same group of animals showed a decline in cortisol levels from the first to the second sample, the results strongly support the interpretation that confinement in the shelter did, in fact, produce an initial cortisol elevation that gradually subsided over days. Still, differences in the populations of dogs under study might well have contributed to the magnitude of some observed effects. Finally, it should be noted that Experiment 1 was conducted in the evening, and Experiment 2 was performed in the morning. The roughly similar levels of plasma cortisol observed for dogs taken directly from their cage during their first three days in the shelter in Experiments 1 and 2 suggests that the heightened cortisol concentrations did not simply reflect a shift in circadian rhythms of HPA activity during confinement.

We believe that our results have implications for the welfare of dogs confined for various reasons. First, they suggest that plasma cortisol concentrations can provide a useful measure of the responsiveness of dogs to this situation. Second, they point to the first several days as critical for interventions designed to reduce stress in public shelters. Third, they argue that further exploration of the effectiveness of human interaction to reduce stress is needed.

Behavior problems during routine separations (e.g., barking or destructive chewing when the owner leaves for work) are common among dogs acquired from shelters (25). Although such behaviors might already be more prevalent among dogs brought to shelters than among the general population of domestic dogs, sheltering itself is likely to contribute to their occurrence. For instance, the experience of separation and exposure to novelty in the shelter might sensitize the dog to such experiences, or enhance the attachment to its adoptive human companion, so that the dog responds disproportionately to later, routine separations (e.g., 25). In recent years, there has been increasing evidence that protracted activation of the HPA system has certain severe detrimental consequences for brain and behavior (21, 22). In humans, it has been hypothesized that early stressors, such as abandonment by attachment figures, may sensitize the HPA axis and related central circuits so that the individual responds with a full stress response to progressively smaller amounts of stimulation—this is thought to commonly underlie the development of major depression with melancholic features (8, 9). It is possible that the abandonment of a pet at a shelter leads to a somewhat similar sensitization process. If so, then the later behavior problems, involving excessive responses to routine separations from the new owner, might also reflect the elicitation of a full stress response by what was previously a subthreshold stimulus.

Many studies with primates and rodents have examined the effect of the presence or absence of various classes of social companions on the activity of the HPA axis (for reviews, see 11, 16, 20). Experiment 2 differs from almost all of those earlier studies in two major regards: (1) the social companion was of another species, and (2) certain prescribed forms of social stimulation were provided by the companion. In our earlier study with dogs, we found that passive interaction with the dog’s human male caretaker eliminated the cortisol response to a novel environment (24). Those results appear best interpreted in the context of the caretaker’s role as a long-standing attachment figure for the dogs. But, this interpretation is irrelevant to the current finding of a differential effect of female and male petters in Experiment 2 because all humans were unfamiliar to the dogs at the outset. Rather, something about either the stimulus complex of the females versus the males, or the manner in which they interacted with the dogs seems to have been crucial. It might be relevant in this regard that dogs have been reported to bite disproportionately greater numbers of male than female humans (3).

One possible explanation for the results of Experiment 2 is that dogs are more easily calmed in the presence of the odor of women as opposed to the odor of men, perhaps related to greater androgen concentrations in men. A second possibility is that the form of dog-petting typically exhibited by females is more soothing than that generally characteristic of men. A third possibility, and one that we consider most likely at present, concerns the specific female petters employed in Experiment 3. These women, but not the men, had extensive experience in training dogs with behavior problems. At the time of the experiment, the petting performed by the women and men appeared at least superficially similar, as did the behavioral reactions of the dogs. Nevertheless, the females may, by virtue of their experience, have been more effective in mitigating plasma cortisol elevations of the dogs. Regardless of which of these or other explanations proves to be right, it should be emphasized that the most significant aspect of the results of Experiment 2 is probably not that the females appeared more effective in moderating the cortisol response of the dogs than did the males, but rather that the dogs seemed to be extremely sensitive to small differences in the quality or style of the humans interacting with them. The challenge posed by this finding is to identify the critical dimension on which males and females differed, so that the effectiveness of human interaction in this situation might be maximized.

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