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Social Dynamics and the Cortisol Response to Immobilization Stress of the African Wild Dog, *Lycaon pictus*

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The aims of the study were to characterize the cortisol response to immobilization stress in African wild dogs (*Lycaon pictus*) and to investigate the relationship between stress and sociality in these pack-living canids. *Ad lib.* observations were made on a captive pack of 19 wild dogs. Individuals were classified as either dominant or subordinate. Cardinal and ordinal dominance indices were also calculated for pack members, as were three other behavioral indices. Active and passive dominance styles were distinguished. Serial blood samples were drawn from animals after chemical immobilization and again after ACTH challenge. The relationship among rank, plasma cortisol concentration, and behavioral style was investigated. When data were combined over the entire study period, there was no obvious relationship between rank and cortisol concentrations or cortisol responsiveness to immobilization stress. Instead, younger animals had higher cortisol concentrations than older ones. Age cohorts were also clearly separated on the basis of behavioral profiles. For males, these distinctions among age classes were especially apparent during the second part of the study period, when subadults occupied dominant positions in the hierarchy. Adult males maintained relatively low cortisol concentrations, despite being involved in and losing a high proportion of dominance interactions. Age-related differences in cortisol profiles of dominant individuals may be explained by differences in the style of dominance employed, with younger males using proportionately more active dominance (threats used to elicit submission). The separation of age classes according to rank, behavioral styles, and cortisol concentrations may reflect improved social skillfulness with age. © 1997 Academic Press

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Animals which belong to social groups are subject to a particular suite of stresses and this may be reflected by concentrations of glucocorticoids in the blood. Increased cortisol concentrations may be associated with social instability (Sapolsky, 1983; Alberts, Sapolsky, and Altmann, 1992; Gust, Gordon, and Hambright, 1993), while the presence of familiar social partners may help to ameliorate the stress response (Levine, 1993; Lyons, Ha, and Levine, 1995). In a number of primate species, an individual's cortisol response to stress is associated with its rank in the social hierarchy (Manogue, Leshner, and Candland, 1975; Golub, Sassenrath, and Goo, 1979; Sapolsky, 1982; Eberhart, Keverne, and Meller, 1983; Welker, Schafer-Witt, and Voigt, 1992). In some species, however, cortisol concentrations appear to be less dependent on rank than on reproductive status (Saltzman, Schultz-Darken, Scheffler, Wegner, and Abbott, 1994), coping styles (Vickers, 1988; Bohnen, Nicolson, Sulon, and Jolles, 1991), or social skillfulness (Sapolsky and Ray, 1989; Sacsher and Lick, 1991).

The African wild dog (*Lycaon pictus*) lives in packs which have well-defined social hierarchies (Frame, Malcolm, Frame, and van Lawick, 1979). The species thus provides a suitable model for investigating the relationship, hitherto virtually unexplored, between stress and sociality in canids. The aim of this study was to characterize the cortisol response of wild dogs to a controlled stressor—chemical immobilization—and to investigate whether this response was related to rank or to style of behavior during social interactions.

METHODS

Study Animals

A captive pack of 19 wild dogs was maintained under seminatural conditions at the Hoedspruit Cheetah Center, 20 km south of the town Hoedspruit in the eastern Transvaal, South Africa. The pack consisted of 9 females and 10 males. These comprised adults (cohort 1) and two generations of offspring (cohorts 2 and 3, collectively referred to as subadults, although by the end of the study period, members of cohort 3 were over 2 years old). The age of pack members ranged from 0.3 to 5.4 years at the beginning of the study. The pack was kept in a 3-ha enclosure in an area of natural bush and hollowed-out termite mounds provided denning sites for breeding. The animals were fed every second day on eviscerated carcasses or cuts of meat, supplemented with minerals and vitamins. A cement dam provided a permanent water supply.

Behavioral Sampling

The pack was observed from a distance of 5–100 m, from a vehicle to which it was already habituated. Observations were made for intermittent periods of no less than 5 days, from first light for 4 hr in the morning and for 3 hr in the afternoon until last light (total of 697 hr of observation). A pilot study was performed using focal animal sampling but thereafter, *ad lib.* sampling (Altmann, 1974) was employed to maximize observations of dyadic interactions.

Aggressive and submissive behaviors were identified following van Hooff and Wensing (1987) and Derix (1994). Agonistic dominance interactions (submission expressed by the subordinate individual in response to a threat from the dominant individual) and affiliative dominance interactions (no threat discernible from the dominant individual but submission by the subordinate) were recorded. Ordinal dominance ranks were assigned on the basis of rank hierarchies, with a rank of ‘‘1’’ assigned to the most dominant individual. Hierarchies were constructed according to the direction of dyadic interactions, so that the number of reversals was minimized. Hierarchies involving six or more individuals were tested for linearity according to Landau’s index, as modified by Appleby (1983). Separate hierarchies were constructed for males and females and new hierarchies were drawn up after each successful challenge for alpha position, resulting in five male (MH1–5a) and four female (FH1a,b, FH2, and FH3) hierarchies. Blood samples were drawn twice during FH1, hence the division of this hierarchy into two parts. MH5 was characterized by an unstable and a stable period but in the present study, blood samples could be drawn only during the unstable period, MH5a.

A cardinal dominance index, I_{DOM}, was calculated as the percentage of all an individual’s interactions which that individual won. Note that if few observations of dominance interactions were recorded for an individual, the assigned cardinal rank could be misleading. Other indices used were an index of wins (I_{WIN}), an index of defeat (I_{DEF}) (the respective number of interactions won or lost by an individual, expressed as a percentage of all interactions recorded during that hierarchy), and an index of involvement (I_{INV}) (all of an individual’s dominance interactions, expressed as a percentage of all interactions recorded during that hierarchy). I_{WIN}, I_{DEF}, and I_{INV} could be affected by observer bias for or against certain study animals but indices calculated for the same animals using both focal and *ad lib.* data were significantly correlated (I_{WIN}: Spearman’s $r = 0.62$, $n = 20$, $P < 0.01$; I_{DEF}: $r = 0.80$, $n = 20$, $P < 0.0005$; I_{INV}: $r = 0.82$, $n = 20$, $P < 0.0005$), indicating that such bias was minimal.

Two styles of dominance were defined according to the presence or absence of aggressive behavior by the dominant individual: passive dominance, expressed during affiliative interactions, and active dominance, expressed during agonistic interactions. A pilot study was performed at the outset of the study and it was found that there was no significant difference in the proportion of passive and active dominance interactions observed using *ad lib.* and focal sampling ($\chi^2 = 3.84$, $df = 1$, $P > 0.1$), indicating that observer bias was once again minimal. Thereafter, *ad lib.* sampling was employed for data collection. Within each hierarchy, the styles of dominance of individuals were examined only for dominant individuals which were involved in proportionately more dominance interactions than expected by chance; e.g., if there were 10 individuals in the hierarchy, only those involved in >10% of interactions were considered. These individuals were characterized according to which of the two types of dominance they used most frequently.

Immobilization and Blood Sampling

Pack members were immobilized several times during the study period ($n = 64$). Darting took place either from a vehicle in the main camp or immediately after wild dogs had been isolated in a smaller feeding camp.

Animals were immobilized with fentanyl (2–2.5 mg/animal) and xylaxine (15–25 mg/animal) (Kyron Laboratories (Pty) Ltd., Benrose, Johannesburg). Fentanyl is an opiate substitute which could influence adrenohypophyseal function and therefore cortisol secretion; however, since the drug and dose used were standardized throughout the study, all individuals should have been affected in a similar way.

Cortisol release in a number of species is subject to an episodic, circadian, and meal-stimulated pattern

(Baxter and Tyrrell, 1987). Food was thus withheld from animals for at least 24 hr before darting and animals were immobilized only between 0930 and 1230. After darting, additional half-doses of drugs were administered as required at about 60-min intervals.

Immobilized animals were removed from the wild dog enclosure and sampling procedures were performed out of sight of the rest of the pack. Blood samples were collected at 10-min intervals for 70 min after darting. Most samples were drawn via a catheter inserted in the jugular vein and saline solution (Plasmalyte-B injection saline; SABEX, I.J. Adcock Ingram, Critical Care Division, Johannesburg) was administered at a rate of approximately 20 drops/min to keep the catheter open. Blood was collected into heparinized tubes. Samples were stored upright at 4°C until centrifugation for 15 min at 3 500 rpm. They were then stored at −20°C until being assayed. After the 70 min of serial sampling, porcine ACTH (40 IU/ml) (Acthar Jel, Fisons Pharmaceuticals (Pty) Ltd., Chloorkop, South Africa) was administered intramuscularly and serial samples were collected at 20-min intervals for a further 2 hr. During 8 immobilization events, individuals received 8 units of ACTH, while during 41 immobilization events, individuals received 16 units of ACTH. On one occasion, two animals were injected with an equivalent volume of saline solution, thereby serving as a control.

After sampling, the effects of the drugs used for immobilization were reversed with yohimbine (0.125 mg/kg) and Narcan (1.2 mg/animal) (Boots Pharmaceuticals (Pty) Ltd., Isando, Johannesburg). This ensured a speedy recovery and minimized disturbance of the pack. Nevertheless, immobilization sometimes precipitated dominance challenges. This occurred regardless of whether treated animals were returned to the pack immediately after administration of the antidote or after being kept overnight in a holding camp. In all except the last male hierarchy (MH5a), darting took place at least 2 months after the last hierarchy change. MH5a represented a transition period when relative rank positions were still unresolved and darting took place only 1 month after the last hierarchy change.

Plasma cortisol was assayed in duplicate using a validated human radioimmunoassay kit (Baxter Travenol Diagnostics CA-529; validation described in de Villiers, Meltzer, van Heerden, Mills, Richardson, and van Jaarsveld, 1995).

The initial cortisol concentration (INIT), measured 7–12 min after darting, was recorded. This was an approximation of basal cortisol concentration. The stress of immobilization probably results from the disorientation prior to unconsciousness rather than the pain of the intramuscular injection (Sapolsky, 1982). Nevertheless, initial samples were usually drawn 5 min after disorientation, therefore cortisol concentrations may have altered from basal levels. Furthermore, anticipatory stress could have caused an alteration from basal levels (Knox, 1992)—different individuals may perceive identical darting situations differently and this could affect initial cortisol concentrations.

The first peak in cortisol concentration after darting (PEAK) was also considered. This was a measure of the maximum secretion of cortisol in response to immobilization. The initial rather than the absolute peak within 70 min of darting was considered, since the former usually occurred within the first 60 min and was thus less likely to be affected by changes in the animals' state of sedation. Nonetheless, the magnitude of the initial peak could be affected by other uncontrolled stressors, e.g., body temperature.

Maximum cortisol concentration recorded within 2 hr of ACTH administration (A-MAX) was thus also recorded. This should be indicative of the size of the adrenal gland and of its responsiveness to ACTH (Baxter and Tyrrell, 1987).

Statistical Analysis

Parametric tests (Zar, 1984) were employed only if variables followed a normal distribution. The indices, I_{DOM}, I_{WIN}, I_{DEF}, and I_{INV}, were percentages and met this criterion after arcsine transformation. Statgraphics (Statistical Graphics Corporation Inc., USA) software (Version 5.0) was used to perform a Principal Component Analysis, following Jolliffe (1986).

RESULTS

The Cortisol Response to Immobilization

None of the three measures of cortisol concentration was significantly correlated with the time of day of darting (INIT: Spearman's $r = -0.15$, $n = 38$, $P > 0.1$; PEAK: $r = -0.04$, $n = 59$, $P > 0.5$; A-MAX: $r = 0.04$, $n = 49$, $P > 0.5$); thus results from across the range of sampling times were assumed to be comparable.

Isolation of animals in the feeding camp allowed swift darting but elicited considerable excitement prior to darting. Animals darted in the main camp appeared far less excited prior to darting but there was often a substantial delay between approach and darting, due to their distrust of a human with a dartgun. Despite the different predarting reactions of animals darted in the main camp and in the feeding camp, there was no difference between the two groups in the three mea-

TABLE 1
Linearity of Male (M) and Female (F) Wild Dog Hierarchies in a Captive Pack, Based on Dominance Interactions

Hierarchy	N	Age of alpha (years)	Duration (months)	d	K	P
(a) Male						
MH1	8	4.6	>1.5	1.0	0.95	<0.006
MH2	8	5.8	3	12.5	0.38	>0.100
MH3	10	6.0	2	14.0	0.65	<0.008
MH4	10	1.4	7	12.0	0.70	<0.008
MH5a	10	1.7	<1	31.0	0.23	>0.500
(b) Female						
FH1a[a]	4	4.5	2			
FH1b	9	1.7	1.5	16.0	0.47	>0.100
FH2	8	2.0	>1.5	17.0	0.15	>0.100
FH3	8	1.7	>1.5	3.75	0.81	<0.006

Note. N, group size; d, No. of circular triads; K, degree of linearity; P, probability of d being significant.
[a] Cannot be meaningfully tested for linearity as $N < 6$.

sures of cortisol concentration (Mann–Whitney; INIT: $Z = 0.81$, $n = 12$, $P > 0.1$; PEAK: $Z = 0.71$, $n = 18$, $P > 0.1$; A-MAX: $Z = 0.05$, $n = 16$, $P > 0.5$). Results were pooled for subsequent analyses.

Disorientation was observed about 5 min after darting and animals were usually fully sedated within 7 min of darting. There was no significant correlation between cortisol concentration and fentanyl dose administered (INIT: Spearman's $r = -0.05$, $n = 38$, $P > 0.5$; PEAK: $r = 0.15$, $n = 59$, $P > 0.1$). INIT and PEAK cortisol concentrations (INIT and PEAK: Spearman's $r = 0.61$, $n = 35$, $P < 0.0005$) and PEAK and A-MAX cortisol concentrations (Spearman's $r = 0.52$, $n = 46$, $P < 0.0005$) were positively correlated. All measures of cortisol concentration were, however, significantly different from one another (Wilcoxon paired samples; INIT and PEAK: $Z = 5.10$, $n = 34$, $P < 0.0001$; INIT and A-MAX: $Z = 4.47$, $n = 26$, $P < 0.0001$; PEAK and A-MAX: $Z = 5.59$, $n = 46$, $P < 0.0001$) and were treated separately in subsequent analyses.

The significant increase in cortisol titers after ACTH administration indicated that wild dogs were responsive to this challenge. ACTH challenge resulted in a 3.06-fold increase over INIT and a 1.28-fold increase above PEAK concentrations. These changes were substantially higher than those recorded for the two control animals, which showed a 1.51-fold decrease and a 1.29-fold increase over INIT concentrations, and a 1.66- and 1.43-fold decrease below PEAK concentrations. There was no significant difference in A-MAX cortisol concentrations of animals which received low and high doses of ACTH (Wilcoxon paired samples; $Z = 1.05$, $n = 8$, $P > 0.1$); thus all results were pooled for analysis.

Age, Rank, and the Cortisol Stress Response

One female and three male hierarchies were significantly linear (Table 1) and for these hierarchies, ranks were assigned to individuals with confidence. Nonlinear hierarchies typically had few observations for some dyads and ranks were then assigned on the basis of relative ranks in previous hierarchies or on the assumption of transitivity. There were significant negative correlations between ordinal ranks and cardinal dominance indices (I_{DOM}) for males (Spearman's $r = -0.812$, $n = 40$, $P < 0.0001$) and for females ($r = -0.781$, $n = 24$, $P < 0.0005$).

During the first part of the study period (MH1–3 and FH1a, b), adults were dominant over subadults but during the latter part (MH4–5a, FH2–3), subadults successfully challenged adults for rank position. This was true for both sexes (de Villiers *et al.*, in preparation) (Table 1).

When the results over the entire study period were combined, none of the three measures of cortisol response was significantly correlated with the ranks of males or females. PEAK values of males and females and A-MAX values of males were, however, negatively correlated with age (Table 2); i.e., younger animals had higher cortisol concentrations (Table 3). The relationship between cortisol concentrations of males and age and rank differed during the first and last parts of the

TABLE 2
Correlations of Age and Ordinal Rank with Three Measures of Cortisol Concentration of Wild Dogs, (A) over the Entire Study Period, (B) over the First Part of the Study Period, When Adult Males Were Dominant over Subadults, and (C) over the Second Part of the Study Period, When Subadult Males Were Dominant over Adults

	INIT	PEAK	A-MAX
(A) Entire study period			
Male age	+0.10	−0.51 (*)	−0.555 (*)
Male rank	−0.21	−0.09	−0.28
Female age	+0.30	−0.47 (*)	−0.18
Female rank	+0.08	+0.05	+0.22
(B) Period 1: Adults dominant			
Male age	+0.16	−0.50 (*)	−0.32
Male rank	+0.22	+0.29	−0.03
(C) Period 2: Subadults dominant			
Male age	−0.54	−0.57 (*)	−0.51 (*)
Male rank	−0.69 (*)	−0.46 (*)	−0.49 (*)

Note. All tests two-tailed Spearman's rank correlation (*, statistically significant, $P < 0.05$).

TABLE 3
Summary Statistics of Cortisol Concentrations (nmol/liter) for Subgroups within a Pack of Wild Dogs (Means ± SD (*n*) [range])

	INIT	PEAK	A-MAX
All pack members	114.72 ± 47.89[a] (37) [47–248]	258.29 ± 48.03[a] (58) [87–351]	335.53 ± 49.27[a] (49) [222–438]
Female	136.32 ± 38.69[b] (15) [56–208]	266.92 ± 54.97 (19) [87–337]	359.51 ± 39.13[c] (19) [299–438]
Male	100.00 ± 48.71[b] (22) [47–248]	254.09 ± 44.42 (39) [178–351]	320.33 ± 49.52[c] (30) [222–419]
Male adults	79.66 ± 21.56 (13) [47–119]	233.20 ± 38.62[d] (20) [178–331]	296.49 ± 44.49[e] (15) [222–367]
Male subadults	129.37 ± 62.35 (9) [58–248]	276.89 ± 42.30[d] (17) [211–351]	344.06 ± 45.02[e] (14) [263–419]

Note. a–e, significant differences ($P < 0.05$) between groups marked with the same symbols (Mann–Whitney).

study period, however. Initially, when adults were dominant over subadults, only PEAK concentrations of males were correlated with age (Table 2) and there was no difference in the cortisol concentrations of adults and subadults (Mann–Whitney; INIT: $Z = 0.47$, $n = 11$, $P > 0.1$; PEAK: $Z = -1.91$, $n = 18$, $P > 0.05$; A-MAX: $Z = -0.63$, $n = 10$, $P > 0.5$). Once subadult males had become dominant, however, both age and rank were significantly correlated with most measures of cortisol concentration (Table 2). During this period, all measures of cortisol concentration were significantly higher for subadults than for adults (Mann–Whitney; INIT: $Z = -2.65$, $n = 11$, $P < 0.01$; PEAK: $Z = -2.08$, $n = 19$, $P < 0.05$; A-MAX: $Z = -2.57$, $n = 19$, $P < 0.05$).

INIT and A-MAX concentrations of females were significantly higher than those of males (Table 3) (Mann–Whitney; INIT: $Z = -2.55$, $n = 37$, $P < 0.01$; A-MAX: $Z = -2.72$, $n = 48$, $P < 0.01$). The cortisol concentrations of adult and subadult females could not be compared during the first and second parts of the study period, as they were for males, since there was only one adult female in the pack for a large part of the study.

There was no significant difference between high-ranking individuals, ranked in the upper half of the hierarchy, and their low-ranking counterparts, ranked in the lower half of the hierarchy, in INIT, PEAK, or A-MAX cortisol concentrations. This was true for both sexes. PEAK cortisol concentrations differed significantly from A-MAX concentrations (Mann–Whitney; $P < 0.05$). Throughout the period of immobilization, all cortisol concentrations (except those of dominant females, measured 30 min after darting; Fig. 1c) were significantly higher than initial values (Mann–Whitney; $P < 0.05$). When comparing consecutive samples, it was evident that cortisol concentrations of high-ranking males increased significantly until 40 min postdarting (Fig. 1a), while those of low-ranking males increased significantly until 30 min postdarting (Fig. 1b). Cortisol concentrations of high-ranking females increased significantly only until 20 min postdarting (Fig. 1c), compared to the significant increase until 30 min postdarting for low-ranking females (Fig. 1d).

Age and Behavioral Profiles

For males, but not females, PEAK cortisol concentrations were significantly correlated with I_{INV} and I_{DEF}

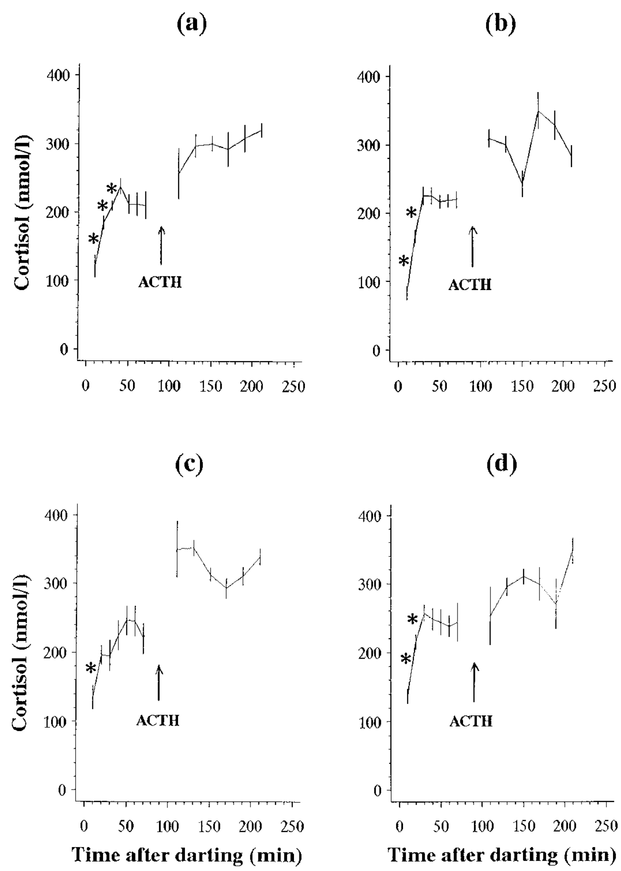

FIG. 1. The cortisol response of wild dogs over 70 min following chemical immobilization and over 2 hr following subsequent ACTH challenge. Means ± 1 SEM of plasma cortisol concentration (nmol/liter). * indicates a significant difference between consecutive values. (a) High-ranking males, (b) low-ranking males, (c) high-ranking females, and (d) low-ranking females.

TABLE 4

Correlations between Age, PEAK Cortisol Concentration, and Several Behavioral Variables (I_{DOM}, cardinal dominance index; I_{INV}, index of involvement; I_{WIN}, index of wins; I_{DEF}, index of defeat) for Wild Dogs

	AGE	PEAK	I_{DOM}	I_{INV}	I_{WIN}	I_{DEF}
AGE		−0.51 (*)	+0.00	+0.74 (*)	+0.34 (*)	+0.56 (*)
PEAK	−0.47 (*)		+0.07	−0.51 (*)	−0.23	−0.40 (*)
I_{DOM}	−0.10	+0.06		+0.21	+0.85 (*)	−0.54 (*)
I_{INV}	+0.23	−0.19	+0.31		+0.62 (*)	+0.55 (*)
I_{WIN}	+0.11	−0.07	+0.79 (*)	+0.48 (*)		−0.12
I_{DEF}	−0.03	−0.24	−0.59 (*)	+0.40	−0.37	

Note. Data combined over the entire study period. Figures for males and females above and below the diagonal, respectively. All tests two-tailed Spearman's rank correlation. (*, statistically significant, $P < 0.05$).

(Table 4). Peak cortisol concentrations, age, rank, and several behavioral indices were all intercorrelated. A principal component analysis was thus performed using I_{DOM} (the ordinal dominance index), I_{INV} (the index of involvement), I_{WIN} (the index of wins), and I_{DEF} (the index of defeat) to determine whether the separation of age cohorts according to endocrine measures was paralleled by behavioral separation.

Principal component analysis using data from the entire study period revealed that slightly different variables described the social dynamics of male and female pack members (Table 5). For both sexes, large contributions were made to the first principal component by I_{DOM} and I_{WIN} but for females, these were strongly contrasted with I_{DEF} while for males, I_{INV} also made a considerable contribution. In the second principal component, the main sources of variation for both sexes were I_{INV} and I_{DEF}. The third principal component made a relatively small contribution to the total variance (3 and 4% for males and females, respectively) (Table 5).

Because of the previously identified difference in the relationship among male age, rank, and cortisol concentration between the first and last parts of the study period (Table 2), we repeated the PCA for each observation period separately. Patterns identified for the entire data set were similar to those for the first part of the study period, when adult males were dominant (Tables 5 and 6). However for the second part of the study period, when subadult males were dominant, the contributions of variables to the total variance was similar to that of females for the entire study period (Tables 5 and 6).

Graphic representation of the variance around the first two principal components revealed that for the entire study period, as for the period when adult males were dominant, male age cohorts were separated along both principal components, although there was some overlap between cohorts (Fig. 2a). When subadults were dominant, there was a clear separation of cohorts along PC-2, with almost no overlap (Fig. 2c). For females, a PCA on data from the entire study period separated the two oldest cohorts from the youngest cohort along PC-2. Apart from one outlier, there was little overlap between the two groups (Fig. 2b).

TABLE 5

Eigenvectors and Eigenvalues for Principal Component Analyses Performed on Four Indices of Social Behavior (arcsine transformation) for Male and Female Wild Dogs and the Contributions of Each to the Total Variation

	Male			Female		
Variable	PC-1	PC-2	PC-3	PC-1	PC-2	PC-3
Eigenvector						
I_{DOM}	0.57	−0.34	0.74	0.66	−0.01	−0.53
I_{INV}	0.47	0.55	−0.22	0.16	0.75	−0.36
I_{WIN}	0.64	0.13	−0.32	0.62	0.23	0.75
I_{DEF}	−0.20	0.75	0.55	−0.40	0.62	0.15
Eigenvalue	2.30	1.55	0.13	2.15	1.62	0.18
% Contribution to total variation	57.57	38.71	3.27	53.79	40.61	4.44
Cumulative	57.57	96.28	99.55	53.79	94.40	98.84

Note. Data combined over entire study period.

TABLE 6

Eigenvectors and Eigenvalues for Principal Component Analyses Performed on Four Indices of Social Behavior (arcsine transformation) of Male Wild Dogs, and the Contributions of Each to the Total Variation

	Period 1: Adults dominant			Period 2: Subadults dominant		
Variable	PC-1	PC-2	PC-3	PC-1	PC-2	PC-3
Eigenvector						
I_{DOM}	0.57	−0.24	0.75	0.65	−0.22	−0.72
I_{INV}	0.52	0.46	−0.42	0.25	0.68	−0.06
I_{WIN}	0.62	0.04	−0.23	0.61	0.33	0.51
I_{DEF}	−0.15	0.85	0.45	−0.38	0.61	−0.47
Eigenvalue	2.60	1.25	0.14	2.06	1.85	0.07
% Contribution to total variation	65.10	31.23	3.52	51.50	46.21	1.82
Cumulative	65.10	96.33	99.85	51.50	97.71	99.53

Note. Data separated for the first part of the study period (Period A) when adult males were dominant, and the second part of the study period (Period B), when subadult males were dominant.

Styles of Dominance and the Cortisol Response to Immobilization

Most classifications of adult males, but only one of subadult males, were as passive aggressors (Binomial test; $X = 8$, $n = 11$, $P > 0.5$; $X = 6$, $n = 7$, $P > 0.2$, respectively). The only adult female which could be classified was a passive aggressor, while all 10 classifications of subadult females were as active aggressors.

The correlation between the percentage of active dominance used and PEAK cortisol concentration of males was not significant. However, PEAK concentrations of active dominants were significantly higher than those of passive dominants (Student's $t = -2.924$, $df = 10$, $P < 0.05$). In an attempt to remove the inherent effects of age from this analysis, we calculated the correlation between the residuals of the regressions of age and PEAK concentration and of age and the percentage of active aggression. The correlation was not significant, indicating that once the effect of age is removed, there is no correlation between style of dominance and PEAK cortisol concentration.

DISCUSSION

The Cortisol Response to Immobilization Stress and ACTH Challenge in Carnivores

In the present study, chemical immobilization of wild dogs and the administration of exogenous ACTH caused a cortisol response which could be useful in determining the effects of social stress in this species. In order to assess the validity of these results, however, they should be compared with those from other studies on carnivores, despite the difficulties presented by differences in sampling protocols.

The investigation of social stress in captive animals can be confounded by the conditions of captivity, which may be stressful in themselves. However, even in the wild the effects of social stresses may be overridden by other influences, for example, food deprivation stress or fear of attack by interspecific competitors. The mean initial plasma cortisol concentration measured for wild dogs in this study was lower than that of another colony of captive wild dogs and of free-ranging wild dogs, using the same sampling protocol (de Villiers *et al.*, 1995). This suggests that the animals used in this study were relatively free from potentially confounding stresses and thus useful for the investigation of social stress. The higher cortisol concentrations of females compared with males may be an effect of oestrogen and oestradiol concentrations, shown to elevate plasma glucocorticoid levels (Saltzman *et al.*, 1994).

The mean initial plasma cortisol concentration (INIT) of wild dogs in the present study was comparable to that of most chemically immobilized carnivores but considerably higher than the basal cortisol concentrations recorded for manually restrained carnivores (Table 7). While it is conceivable that wild dogs have relatively higher basal cortisol titers than the species examined in the latter studies, it is also possible that by the time that initial samples were drawn after chemical immobilisation, cortisol concentrations of wild dogs had already increased above basal levels. In the present study, therefore, INIT cortisol concentration was only an approximation of basal titers.

The mean maximum cortisol concentration (A-MAX) of wild dogs following injection (intramuscular) with Acthar Jel was lower than that recorded for healthy

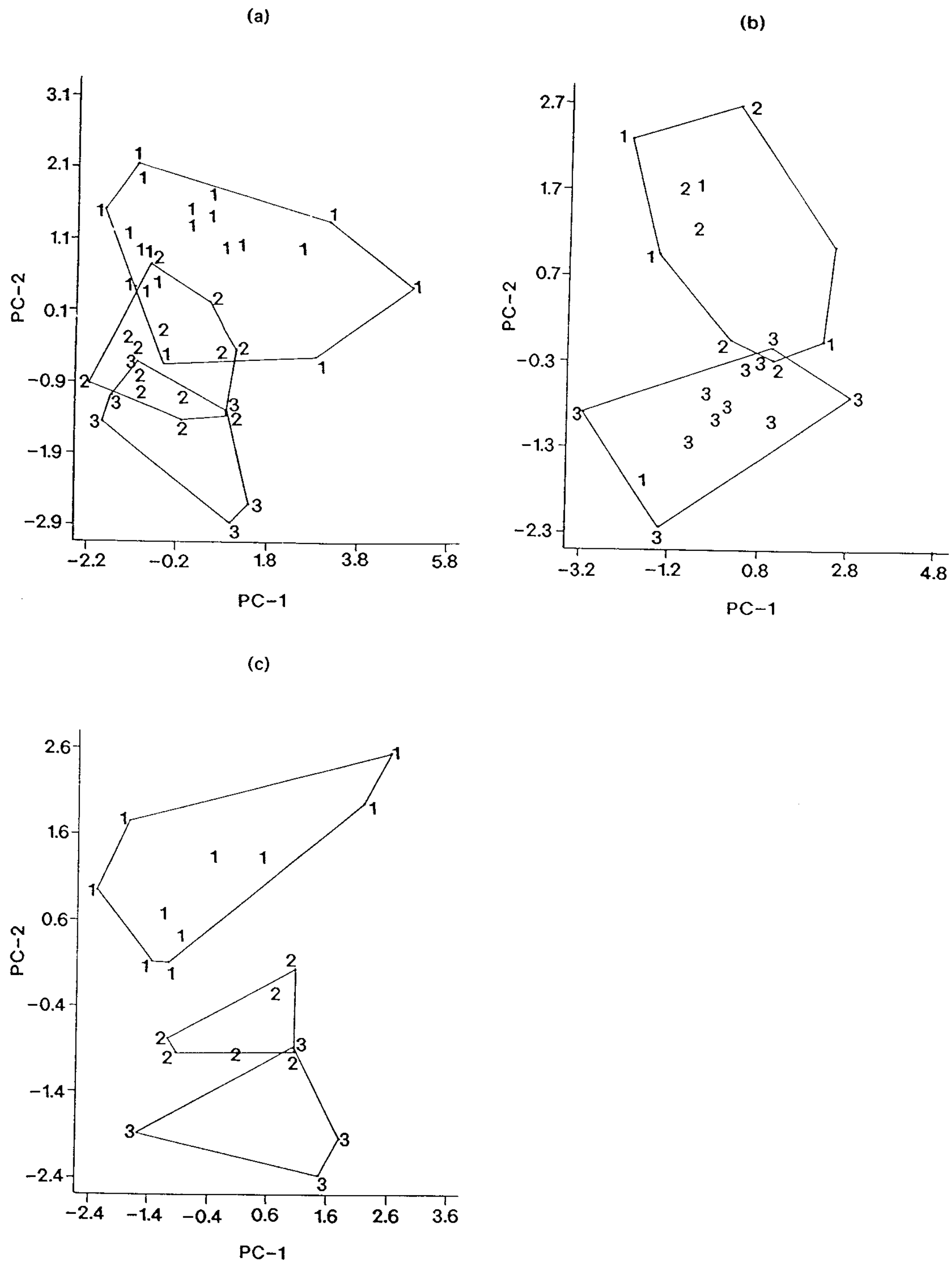

FIG. 2. Graphical representation of the variance around the first two principal components of the PCA performed on four behavioral indices (I_{DOM}, I_{INV}, I_{WIN}, and I_{DEF}). (a) Males, combined over the entire study period, (b) females, combined over the entire study period, and (c) males, combined over the second part of the study period only. 1, cohort 1; 2, cohort 2; 3, cohort 3. Groups of individuals which provide the most resolution are included in convex hulls.

domestic dogs (and considerably lower than that of domestic dogs with hyperadrenocorticism) following administration (iv) of α^{1-24} ACTH. It was, however, similar to that of ferrets (*Mustela putorius furo*) injected (im) with cosyntropin (Table 7). A lack of effect of ACTH dose on the magnitude of the cortisol response was also reported for ferrets (Rosenthal, Peterson, Quesenberry, and Lothrop, 1993), deer (*Cervus elaphus* and *Dama dama*) (Bubenik and Bartos, 1993), and humans (Crowley, Hindmarsh, Honour, and Brook, 1993) and indicates that the administration of the low ACTH dose was sufficient to stimulate a maximum cortisol response in the present study.

PEAK cortisol concentrations were significantly

TABLE 7

Basal/Initial Cortisol Concentrations, and Cortisol Concentrations Following ACTH Administration, of Manually Restrained (Manual) and Chemically Immobilized (Chemical) Wild (W) and Captive (C) Carnivores

Restraint	Species	W/C	Description	Initial/basal conc (nmol/l)	Conc after ACTH (nmol/l)	Reference[a]
Manual	Domestic dog (*Canis domesticus*)	C	Healthy	65 ± 22		1
		C	Nonadrenal illness	158 ± 70		1
		C	Healthy	150 ± 70	510 ± 130	2
		C	Adrenal illness[b]	330 ± 100	17900 ± 760	2
	Ferret (*Mustela putorius furo*)	C		74 ± 7	303 ± 50	3
	Dingo (*Canis familiaris dingo*)	C	Males:			
			breeding	143 ± 36		4
			nonbreeding	25 ± 6		
			Females:			4
			breeding	81 ± 25		
			nonbreeding	31 ± 8		
Chemical	Wolf (*Canis lupus*)	C		41–126 (range)		5
	Spotted hyaena (*Crocuta crocuta*)	W, C	Male	164 ± 28		6
			Female	168 ± 40		
	Wild dog (*Lycaon pictus*)	W		144 ± 76		7
		C		180 ± 41		7
		C		114 ± 48	336 ± 49	8

[a] [1]Church *et al.* (1994); [2]Meijer *et al.* (1978); [3]Rosenthal *et al.* (1993); [4]Corbett, 1988; [5]Packard *et al.* (1985); [6]van Jaarsveld and Skinner (1992); [7]de Villiers *et al.* (1995); [8]this study.

[b] Hyperadrenocorticism.

correlated with A-MAX concentrations and it was assumed that the former, like the latter, was a measure of the maximum secretory ability of the adrenal gland.

Social Status and Stress in Wild Dogs

In several species, particularly primates but even reptiles (Greenberg, 1990), social status is associated with the concentration of circulating glucocorticoids. Dominant individuals tend to have lower basal cortisol concentrations than subordinates (Manogue *et al.*, 1975; Sapolsky, 1982; Welker *et al.*, 1992). This may be because dominant individuals experience lower daily levels of stress, receiving less aggression (e.g., Sapolsky, 1982; Eberhart *et al.*, 1983) and more friendly gestures (e.g., Seyfarth, 1976) than subordinates and being assured of access to limited resources such as food (e.g., Frank, 1986) and mates (e.g., Bulger, 1993). The lower basal cortisol concentrations of dominant male olive baboons (*Papio ursinus*) were also associated with greater stress responsiveness, i.e., a relatively greater and faster increase in cortisol concentration following application of a stressor (Sapolsky, 1982).

Within a wild dog pack, there are separate and often linear male and female hierarchies, headed by a dominant or alpha pair (Frame *et al.*, 1979). Relative dominance positions within dyads are usually well-defined: dominance is sometimes enforced by aggression although it is often acknowledged by subordinates in the absence of aggression. Younger pack members typically have preferential access to food, but rank is important in determining reproductive opportunities (Frame *et al.*, 1979; Reich, 1981). The rank-related differences in cortisol profiles which occur in other social species might thus be expected to occur in wild dogs.

In the present study, when data were combined over the entire study period, there was no obvious relationship between rank and cortisol concentrations or cortisol responsiveness to stress. This was so, regardless of whether individual cardinal or ordinal ranks or coarse rank category was considered. Instead, cortisol concentrations were negatively correlated with age. The difference in cortisol concentrations between subadult and adult males was especially apparent during the second part of the study period, after subadults had successfully challenged for dominant rank positions. During this period, cortisol concentrations were negatively correlated with both rank and age; i.e., younger, dominant individuals had higher cortisol concentrations than older, subordinate individuals.

Older domestic dogs had higher basal and peak cortisol concentrations than younger animals (Strasser, Niedermuller, Hofecker, and Laber, 1993; Rothuizen, Reul, van Sluijs, Mol, Rijnberk, and de Kloet, 1993). The opposite pattern found in the present study on wild dogs cannot, therefore, be explained as an effect of the maturation of the hypothalamo-pituitary-adrenal axis. Furthermore, if the latter explanation were correct, differences in cortisol concentrations should have been less rather than more pronounced in the second part of the study period, since most subadult males were then over 2 years old and thus likely to be as sexually mature as adults. (Female wild dogs are sexually mature at 23 months (van Heerden and Kuhn, 1985) and male and female wolves at 2 years of age (Schotté and Ginsburg, 1987)).

Another possible explanation for the age-related differences in cortisol concentrations of wild dogs is that these concentrations are influenced by social skillfulness, which improves with age.

Social Skillfulness and Stress in Wild Dogs

Several authors (Yodyingyuad, Eberhart, and Keverne, 1982; McGuire, Brammer, and Raleigh, 1986) found no relationship between rank and cortisol concentration and it has been suggested that social skillfulness may be a better predictor of the stress response than rank. A critical feature of psychological stress is predictability and the ability to assess potentially stressful situations and react appropriately may be reflected in an animal's cortisol profile (Sapolsky and Ray, 1989). In humans, a significant correlation existed between cortisol concentrations and a clinical rating of the effectiveness of psychological defenses against stress (Vickers, 1988) as well as certain coping styles (Bohnen *et al.*, 1991). For male olive baboons (*Papio anubis*), low basal cortisol concentrations were associated with particular styles of dominance involving a high degree of social skillfulness, control, and predictability over social contingencies. Dominant baboons lacking these behavioral features were found to have cortisol levels as high as subordinates (Sapolsky and Ray, 1989). Rearing conditions appeared to influence the social skillfulness and associated concentrations of glucocorticoids in the blood of male guinea pigs (*Cavia aperea f. porcellus*) (Sachser and Lick, 1991).

If social skillfulness in wild dogs improves with age and if a lack of such skillfulness causes stress during social interactions, we would expect subadults to have higher cortisol concentrations than adults, the difference between the two age groups to become more pronounced as competition for rank in the hierarchy becomes more intense, and age-related differences in cortisol profiles to be reflected by differences in behavioral profiles. In the present study, all three predictions held true for male wild dogs, while the first and last held true for female wild dogs (the second prediction was not testable due to small sample sizes).

Surprisingly, adult male wild dogs maintained low cortisol concentrations in spite of being involved in and losing a high proportion of dominance interactions. Such individuals might have been employing a dominance style which the PCA was unable to identify. Stylistic differences in the intensity or severity of aggression have been observed for wolves (Fentress, Ryon, McLeod, and Havkin, 1987) and we therefore investigated the relationship between two styles of dominance and cortisol concentrations of wild dogs.

Passive dominance implies confidence by an individual in its social status since very little, if any, threat is needed to elicit submission from a subordinate. Active dominance, on the other hand, implies insecurity regarding social status, since threats are employed to elicit submission from a subordinate. The higher peak cortisol concentrations of male wild dogs employing active dominance were an indication that social insecurity may be associated with elevated stress levels. Younger males tended to use proportionately more active than passive aggression in dominance interactions than older males, while all subadult females were classified as active aggressors. The relationship between dominance style and cortisol concentration existed only while age was included in the analysis, supporting the hypothesis that social confidence improves with age.

In conclusion, while the cause of variation in the cortisol response of wild dogs to immobilization stress remains speculative, it may be due to individual differences in perceptions of dominance interactions. Social skillfulness probably improves with age so that older, more experienced animals find dominance interactions more predictable and hence less stressful than younger animals. This may become especially evident once subadults must maintain high-ranking positions in the social hierarchy. The social insecurity of young individuals may be reflected by their reliance on a dominance style which employs threats to elicit submission from subordinates. In order to examine this hypothesis rigorously, however, a similar study should be conducted on other packs, controlling for the effects of age and social upheaval.

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