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Cats temper rating score correlate with behavior and adrenocortical reactions to minor stress in cats

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Abstract
In order to better identify, differentiate, and treat stress-related illnesses or behavioural issues in cats, there is an increasing demand for a simple way to measure their stress levels. So, we looked into the potential behavioural changes that could be linked to an adrenocortical reaction to a sudden stress or (shower bath), and how much this response varies depending on the cat's disposition. A feline temperament profile (FTP) was administered to eight male cats to evaluate their behavioural reactions in various test scenarios. An ACTH stimulation test was used to assess the adrenal glands' physiological activity in all of the cats. The felines were surgically implanted with a venous access port system to allow for painless and objective blood collection. Following their recuperation, the cats were subjected to a three-minute spray bath as part of a stress test. Hemochromatometric profiling of cortisol was performed on blood samples taken both before and after the bath. We recorded the cats' every move as they went through the stress test. Researchers looked for associations between FTP scores and adrenocortical and behavioural response markers. While other behaviours remained unchanged, the cats' grooming behaviour increased following the stressor ($p<0.05$). The levels of circulating cortisol in all cats were considerably elevated by the spray bath; these levels peaked fifteen minutes after the stressor was exposed. During the spray bath, cats whose plasma cortisol levels were high at peak times were found to vocalise more (rs = 0.93; $p<0.001$) and display less movement (rs = −0.74; $p<0.05$). There was no correlation between FTP scores and adrenocortical responses. These findings suggest that the cats' individual vocalisation and movement responses to the stressor spray bath were correlative with the plasma cortisol levels minutes following its application. Under these circumstances, the FTP test failed to foretell the behavioural and adrenocortical reactions in anxious cats.

Keywords: Cat temperament, stress response, cortisol, ACTH test, feline behaviour, adrenocortical reaction, FTP score, spray bath stressor.

1. Introduction
Among Asian and Eastern pet owners, felines have recently enjoyed unparalleled popularity. Problems related to their well-being and conduct have so garnered more focus (Bradshaw *et al*., 1999; Rochlitz, 2005) [3, 39]. There are a number of hypothesized physiological and behavioral markers that cats might exhibit when they are stressed. In terms of the welfare recipient's condition (Carlstead *et al*., 1993; Dybdall *et al*., 2007; McCobb *et al*., 2005) [8, 10, 28]. The necessity for sensitive ways of assessing stress-related behaviour is acknowledged by researchers and veterinarians who are concerned with the care and welfare of animals. In addition, stress evaluation is highly beneficial in behaviour therapy and treatment for cats. For a long time, it has been known that stress can cause behavioural issues like elimination problems (inappropriate elimination or urine marking), anxiety or fears and medical issues (Buffington, 2002; Cameron *et al*., 2004; Westropp *et al*., 2006) [7, 5, 49] in cats. Both short-term exposure to a single stressor, known as acute stress, and longer-term exposure to multiple stressors, known as chronic stress, can have negative effects on an organism (Moberg, 2000) [9]. When it comes to short-term stress, physiological assessments may be more accurate, but when it comes to long-term stress, both physiological and behavioral changes can be affected. An increase in urine cortisol and an increase in behaviours like hiding or trying to hide were observed in cats whose caretakers experienced chronic stress due to a change in routine and who were not petted or spoken to as much (Carlstead *et al*., 1993) [8]. Cats also appeared to be less energetic and less likely to play or explore (Carlstead *et al*., 1993) [8], and they stayed

awake for longer periods of time. In a number of domestic and laboratory animal species, glucocorticoid (GC) levels have been utilized as markers for the acute phase stressor's impact and intensity (Pignatelli *et al*., 1996) [33]. Generally speaking, the hypothalamic-pituitary-adrenal (HPA) axis is activated in response to both positive and negative stimuli. This activation improves coping with the stressor by increasing the adrenal gland's GC synthesis and secretion as well as by triggering behavioural and peripheral changes like metabolic alterations or cardiovascular tone. GCs enable, stimulate, or suppress a continual stress response, which in turn allows organisms to adapt and cope with unpredictable conditions (Sapolsky *et al*., 2000; Mormède *et al*., 2007) [42, 32]. Absolute plasma concentrations of GC differ between species and individuals, and the mechanisms controlling its production and secretion are intricate (Romeo, 2004; Mormède *et al*., 2007) [40, 32]. Cats' cortisol levels have been extensively studied and found to be a valid indicator of physiological stress (Carlstead *et al*., 1993; Willemse *et al*., 1993; Rand *et al*., 2002) [8, 50, 35]. This is based on previous research by Kemppainen and Peterson (1996) [20]. The adrenal glands secrete glucocorticoids in pulsatile patterns (Kemppainen and Peterson, 1996) [20], and age (Grewing, 2004; Javadi *et al*., 2004) [15, 17], body weight (Schoeman *et al*., 2000) [43], and seasonal changes (Randall and Parsons, 1987) [36] are among the numerous factors that affect glucocorticoid levels, further complicating interpretation. Cook *et al*. (2000) [9] and Romeo (2004) [40] found that animals undergo stress reactions during the majority of sample techniques. In response to stress, there may be both behavioural and physiological changes. Species- and individual-specific differences in behavioural responses to stress are well-documented (Moberg, 2000) [9]. According to Beerda *et al*. (1998) [2] and Rushen (2000) [41], these reactions can provide valuable information about stress. Some research (Morrison *et al*., 2007; Reynolds *et al*., 2007) [30, 38] used stress-related behavioural ratings to evaluate the cats' cooperation during blood drawing. These scores included things like vocalisation, struggle, withdrawal movement, avoidance behaviour, scratching or biting behaviour, and more. Katzer and Turner (1997) [21], McCobb *et al*. (2005) [28], and Dybdall *et al*. (2007) [10] utilised the Cat-Stress-Score to assess the stress levels of cats in shelters. The score is based on the cats' body language, facial expressions, vocalizations, and activity levels. Nevertheless, McCobb *et al*. (2005) [28] found no evidence of a direct relationship between the Cat-Stress-Score and physiological measures like cortisol levels, which are markers of behaviour. The feline temperament profile (FTP) was tested for validity by Siegford *et al*. (2003) [44]. The FTP is a test for evaluating a cat's temperament that is based on the model created by Lee *et al*. (1983) [24]. They compared the FTP results to the conduct of cats towards familiar and new people, after measuring their responses to standardized scenarios. Positive responses to both known and unknown people are correlated with "acceptable" FTP scores (the number of positively predicted reactions of the cat), according to the study's results. This is where the significance of people's social interactions and handling comes into play. One year after being touched, cats that had been socialised between 2 and 12 weeks of age were quicker to approach, touch, and rub both familiar and new test subjects, according to McCune (1995) [29]. Because the researcher is already familiar with the cats, this impact is more likely to be beneficial in a laboratory context. Siegford *et al*. (2003) [44] also found that FTP scores were stable for at least eight months. Research on dogs (Svartberg *et al*., 2005) [45], domestic cats (Turner *et al*., 1986; Reisner *et al*., 1994; Lowe and Bradshaw, 2001; Siegford *et al*., 2003) [46, 37, 26, 44], and dairy goats (Lyons *et al*., 1988) [27] has shown that temperament is largely concerned with fairly consistent individual traits that exhibit some consistency over time and across contexts. Stressed cats appear to respond differently based on their "personality" and "temperament," suggesting that individual variances may impact the behavioural reactions to a stressor. Inhibited cats, for instance, may have different pituitary-adrenal responsiveness if they are more frightened in reaction to many stimuli, display protective behaviour, or stay motionless (Adamec and Stark-Adamec, 1989) [1]. It has been observed in numerous animal investigations that animals exhibiting shyness and fear exhibit a greater basal cortisol content (for a comprehensive review, see Budaev, 2000). Evidence of elevated blood cortisol levels has been found in a variety of animals, including goats, rhesus monkeys, and cattle that display symptoms of anxiety (Lyons *et al*., 1988; Kalin *et al*., 1998; Bristow and Holmes, 2007) [27, 19, 4]. More agonistic behaviours were associated with elevated cortisol levels in free-roaming, intact female cats, according to a recent study by Finkler and Terkel (2010) [13]. Consequently, this study set out to do two things: first, to find potential behavioral indicators linked to stress responses in cats; and second, to investigate the relationships between temperaments as measured by FTP scores and changes in behavioral and physiological measures in response to a mild stressor.

2. Methods and Materials

2.1 Research methodology

The current investigation lasted for three months. At the beginning of the first day, every cat was given a feline temperament profile (FTP) test. The cats were then randomly tested for appropriateness (sufficient adrenocortical function) using an ACTH stimulation test within the following two weeks. A vascular access port was inserted at random after these tests so that blood samples could be taken without causing any discomfort. The insertion of port catheters in all of the cats took two weeks to finish for pragmatic reasons. At least twelve days following implantation, the cats were subjected to a gentle stressor in the form of a spray bath. The stress test was administered to each cat on a single day at random. According to Az. 66.2-3-22- 2471-232-09, the Iraqi government in Baghdad authorized the animal experiment.

2.2 Pets

This study utilized eight male cats of mixed breed, with short fur and a weight ranging from 4.4 to 5.9 kg (mean 5.0 kg), all of which were neutered. All of the cats were 2.75 years old when the study began; they were all raised in the same facility with consistent handling standards. During the initial four weeks, they were kept in pairs of cage chambers measuring 2.5 m × 3.5 m. The felines were free to roam the room and play with one another. The cats had a surgical procedure to implant a vascular access port (VAP; UNO, Roestvaststaal B.V., The Netherlands) into their left external jugular vein while under anesthesia. This was done in preparation for the stress test, also known as the spray bath. The cats were kept separately in polystyrene cages that measured 70 cm × 90 cm × 80 cm after the VAP was implanted. Each cage also had a wooden shelf that measured 25 cm × 35 cm and was placed in the centre of one side of the cage. In order to keep the catheter open after the first week of implantation surgery, a Huber needle and 5-milliliter syringe were used to flush 2 millilitres of saline solution and 2 millilitres of locking solution (100 IU/ml heparinised saline). Every three to four days and after each blood sample, the catheter was flushed in the second week after implantation procedure. Furthermore, each cat was handled once daily in a manner that would acclimatise them

to the manipulation required for blood sampling. The temperature of the room was maintained at 19±2 ◦C, and there was a 12-hour light cycle and a 12-hour dark cycle. Every morning at 8:00 a.m., the animals received their commercial dry meal for the day, and they had access to water at all times.

2.3 Assessment of cat personality traits

The Feline Temperament Profile (FTP) test, which was administered to all of the cats, took around half an hour to finish. One cat was randomly selected and placed in this test. Opposite the cage, in a room that was completely devoid of people. So that it could adjust to its new home, the cat was given plenty of time. The cat finally calmed down and began to sniff around after about five minutes. The FTP test was initiated by this. There were ten distinct scenarios that made up the test.

1. The first step is for the tester to get down on one knee, perhaps five or six feet away, and to repeatedly cry out to the cat. A hand is outstretched.
2. A second strategy is to call out to the cat again after three feet if it still doesn't come when called.
3. **Amiability:** after approaching or coaxing the cat to come, the tester reaches out a hand (squatting so the hand is below the cat's head) to the feline.
4. **Interaction:** In the course of their conversation, the tester starts to pet the cat all over, including its head, back, and sides. Whatever the case may be, the test must not proceed if interaction cannot be begun within 10-15 minutes.
5. To start playing, the tester steps away from the cat and slowly rolls a ball or string across the floor.
6. **Sociability I:** The tester gently approaches the cat or calls it repeatedly until it comes closer.
7. After reassuring the cat with further strokes, the tester carefully lifts it up and holds it close to his chest till it is calm.
   Sitting down on a chair, the tester sits the cat on his lap so it faces him, and then strokes the cat as part of Sociability II.
8. **Flexibility:** the examiner sets the cat down on the floor beside the chair and uses hand gestures and calls to stimulate it.
9. **Fear or aggression I:** the examiner plants the cat on the floor, firmly grasps its tail, and pulls steadily.
10. **Fear or aggressiveness II:** while the cat is looking away, the tester sets the cat on the floor (not on carpet) and drops a metal box or other object behind it.

According to Siegford *et al*. (2003) [44], the cat's responses were recorded. For each test circumstance, the same individual would administer the FTP test, while a second observer would mark the cat's reactions as "acceptable" or "questionable" (for a complete list of replies, see Table 1). Lastly, for a "acceptable score" (FTP+) and a "questionable score" (FTP−), the total was determined by adding the occurrence (+1) or absence (0) of each stated response, respectively. Twenty healthy laboratory cats were temperament tested before this study for training and validation purposes. The results showed that the FTP− score had an inter-observer reliability of 92% and the FTP+ score of 88% for the two observers.

Table 1: Criteria for determining which FTP test answers are acceptable and which are cause for concern

Test Situation	Acceptable Responses	Questionable Responses
Initial Approach	Direct eye contact, clear voice Slowly approach Rolls, submissively Shows up, takes a whiff of the hand	Keeps their distance, takes a protective stance, and stays out of eye contact Keeps a safe distance from you while watching
Follow Up Approach	Direct eye contact, clear voice Slowly approach Rolls, submissively Shows up, takes a whiff of the hand	Keeps their distance, takes a protective stance, and stays out of eye contact Keeps a safe distance from you while watching
Friendliness	Inhales deeply Presses head into palm, licks and rubs body against palm Submissively, vocalisation rolls	Retreats or adopts defensive position, Throws a punch or makes a threatening gesture, Mouth bites or attempts
Interaction	Grazes the skin of your hand or legs Cat starts making noises that sound like chirping, purring, or meowing. Bumping heads Thorns carefully encircle you Arrives frightened but soon calms down	Taking a position that is aggressive or protective, Strikes out or tries to strike with paw, Makes an effort to bite or actually bites
Play Initiation	Returns to receive additional caresses, Observes the string meticulously, Strikes a chord	Turns away from the string, looks away from the room, and doesn't make eye contact.
Sociability I	Gently looks you in the eyes, then reaches out a paw to pat you on the shoulder or neck.	Fights for freedom, Strikes out or tries to strike with paw, Try biting or actually does bite
Sociability II	Plays with its owner's hand, purring and rubbing gently. Engaging in direct eye contact, Moves in a submissive manner, Raised to sniff the air, puts paw on neck	Resting anxiously on the lap, Descends, becomes hostile (biting or scratching), or threatens
Adaptability	Jumps up Maintains eye contact while rooted to the floor, Mounts the hindquarters and establishes touch	Pays little attention to the call or the tester, then walks away
Aggressiveness or Fear I	Mooches along without making a sound Struggles or tries to get away	Trys to hit hand, makes noises
Aggressiveness or Fear II	Arouses fear, but soon calms down Not concerned with the background noise	When startled, the animal may adopt a protective or aggressive stance before fleeing for cover. Seems oblivious to the sound (have vet examine hearing).

2.4. Stimulated ACTH assay

To make sure the cats' adrenal glands were working normally, we measured their plasma cortisol responses to synthetic ACTH. After drawing blood (2 ml) from the saphenous vein at 12:30 h, two further samples were drawn 45 and 90 minutes later, and ACTH (0.125 mg Synacthen) was injected intramuscularly right after (for the blood sampling schedule, see Fig. 1).

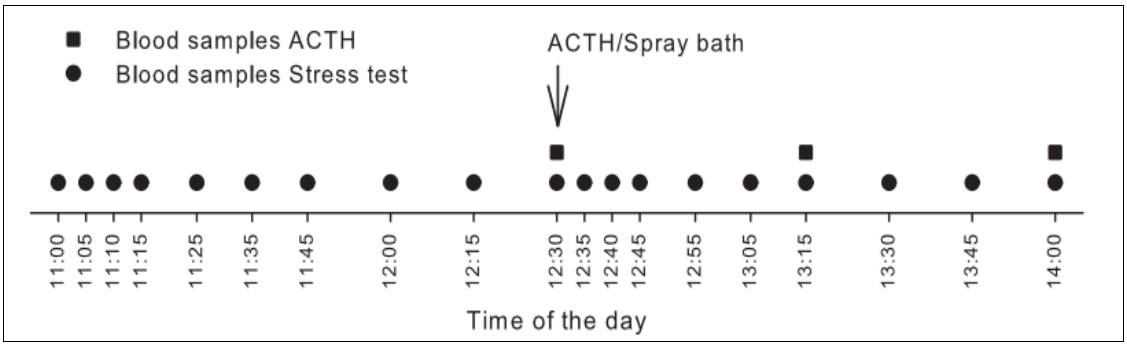

Fig1: Procedure for collecting blood samples during the ACTH stimulation test and the stress test.

2.5 Experiment for measuring stress

At 11:00 a.m., the stress test began and continued for 180 minutes, ending at 14:00 a.m. Separated by the start of the spray bath at 12:30 h, the test had two similar phases with respect to blood sampling and behavioral analysis.

2.5.1 Methods for collecting blood samples and measuring cortisol levels throughout the experiment

One individual kept the cat in his lap without actively restraining it. If the cat was finicky during the blood sample, an aide would give it snacks to calm it down. It was at 11:00 h (t=0) that sampling began. Consistent with the blood sampling schedule shown in Figure 1, a single individual would collect 4.5 ml of heparinised polypropylene tubes containing blood samples using the VAP system. Immediately following the collection of the 90-minute sample (12:30 h), the cat was confined to a cage measuring 30 cm × 30 cm × 45 cm. It was subsequently relocated to the bathing area and spritzed with water at 22 ◦C for a duration of 3 minutes. Therefore, ACTH and the spray bath were administered simultaneously throughout the day. Following the spray bath, the cat was promptly returned to the test subject's lap and delicately dried with a towel for thirty seconds. The same protocol as prior to the spray bath was then used to collect blood samples. The blood samples were spun at 2000 × g for 10 minutes at 4 ◦C as soon as they were collected. In the meantime, portions of plasma were transferred to crushed ice and kept at -80 ◦C until hormone measurements could be taken. A method published by Ling and Jamali (2003) [25] was used to quantify the concentration of cortisol using modified high performance liquid chromatography (HPLC).

2.5.2 Assessments of behavior

Using a video camera (Panasonic NV-GS37EG), each cat was recorded throughout the whole 180-minute stress test. The software "The Observer 4.0" (Noldus Information Technology) was used to analyse behaviour through behaviour sampling with continuous recording. Table 2 displays the definitions of the behaviours that were observed before and after the stressor, including resting, eating treats, grooming, investigatory, avoidance, and aggressive conduct. For definitions, see Table 2. During the 3-minute spray bath, the only behaviours that were measured were movement and vocalisation. The investigation did not include behaviours that were hard to evaluate due to their invisibility. The behavioural metrics were presented as a proportion of the total time observed before and after the stressor. The amount of time spent moving and making noises was measured in seconds. Each cat in the study was evaluated by the identical observer. By analysing the same videos of the two cats multiple times, we were able to determine that the behavioural measurements were reliable (91% intra- and 89% inter-observer reliability) across all behavioural categories.

Table 2: Characterizations of stress-related actions

Behaviors	Definitions
Contact attentions	The feline examines the person doing the experiment by sniffing them or rubbing against them in any way.
Grooming	A cat may lick or chew on its own flesh, or it may use its front paws to dab its head with saliva.
Resting-sleeping	Cat lies or sits calmly, making just small motions with its head and limbs.
Eating treats	Cat eats food treats.
Avoidance behaviors	Without making any effort to flee, the cat displays a frightened reaction by opening its eyes wide, dilating its pupils, or pressing its ears flat against its head.
Investigatory	Cats sit or stand while making eye and head movements to indicate their attentiveness to their surroundings.
Aggressive behaviors	The cat is unpredictable and even tries to bite or scratch the person doing the experiment.
Other	Any behavior, which is not available to judge due to lack of visibility.
During the three minutes spray baths	
Vocalizations	A wide variety of sounds (meows, yowls, growls, etc.) produced by cats.
Locomotions	Whenever the cat moves its head in a different direction within its cage.

2.6. Data analysis with statistics

Microsoft Excel 2013 and the statistical application SPSS were used for the analysis. A one-way repeated measures ANOVA was used to compare the cortisol concentrations in the ACTH and the stress test. When asked, we utilised the Student-Newman-Keuls multiple comparison approach to identify distinct sampling points. To compare the behavioural parameters before and after the stressor, the Wilcoxon signed rank test was utilised. We used Spearman's rank correlation test to look for connections between our physiological and behavioural metrics. We regarded a P-value less than 0.05 to be statistically significant.

3. Result

3.1 Cat personality assessment

You can see the FTP scores of all the cats in Table 3. Most of the time, cats showed responses that were satisfactory. The only test situations in which replies were dubious were 6, 7, and 8. The FTP+ score varied from 6 to 12 (8.0±0.7), while the FTP− score varied from 0 to 3 (1.9±0.4; mean ± SEM; n = 8). The FTP− score and the FTP+ score were significantly inversely correlated (rs = −0.93, p<0.001; Table 4).

Table 3: Results of the cats temperament profiles, the ACTH stimulations test, and stress tests including cortisol concentration in nmol.L^{-1} and durations in seconds of movement and vocalisations during the spray bath.

	ACTH			FTP		Stress tests						Behaviors	
Cats	0	45	90	+	-	90	95	100	105	115	180	voc	loc.
6566	22.8	270.1	209.6	8	2	36.8	159.1	210.1	309.5	239	68.6	51.2	3.9
6564	68.1	228.4	272.5	9	0	43.7	42.6	234.9	318.2	251.7	173.1	3.6	28
6563	39	321.3	341	7	2	85.1	95.7	183.6	174.6	201.5	46.1	16.4	2.7
6559	53	174.8	316.2	12	1	112	149.2	217.9	246.7	197	79	8.2	4.1
2128	24.5	237.1	missing	9	2	87.6	85.7	228.4	230.2	243.1	55.7	10.9	3.8
2127	39.2	263.8	316.1	6	3	17.5	47.1	202.1	271.4	190.8	30.4	49.1	3.1
2125	51.7	201.5	214.7	6	3	18.4	58	176.9	194.6	176.5	15.9	0.5	26
2120	62.6	276.6	408.1	7	2	93.1	159.4	225.1	212.7	115.6	48.3	10.2	8.2

Table 4: Connections between the metrics shown in Table 3.

	ACTH			FTP	Stress tests						Behaviors	
Cats	0	45	90	-	90	95	100	105	115	180	Voc.	loc.
FTP (+)	0.4	-0.39	-0.01	-0.91	0.73	0.31	-0.03	0.23	0.13	0.16	-0.3	-0.03
FTP (−)	-0.1	0.21	-0.06		-0.3	-0.25	0.11	-0.28	-0.3	-0.28	0.26	-0.09
ACTH (0)		-0.44	-0.12		-0.4	-0.41	-0.27	-0.44	-0.4	-0.24	-0.2	0.22
ACTH (45)			0.37		-0.7	0.15	0.33	0.14	0.22	0.33	0.45	-0.34
ACTH (90)					0.35	0.53	0.24	0.27	0.26	0.52	0.57	-0.21
Stres test (90)						0.84	0.15	0.37	0.14	0.49	0.27	-0.43
Stres test (95)							0.44	0.52	0.23	0.37	0.19	-0.57
Stres test (100)								0.23	0.53	0.17	0.53	-0.42
Stres test (105)									0.91	0.85	0.44	-0.3
Stres test (115)										0.94	0.55	-0.18
Stres test (180)											0.8	0.9
Behaviors loc.											-0.93	

3.2 Stimulated ACTH assay

The mean±SEM cortisol value at baseline was 43.7±5.8 nmol/L, with n = 8. Following the delivery of ACTH, the concentration of cortisol sequentially rose to 244.4±15.4 nmol/L at 45 minutes (p<0.05) and then to 305.7±27.6 nmol/L at 90 minutes (p<0.05) in comparison to both the baseline and 45 minutes (Table 3). Consequently, serum cortisol levels increased relative to baseline by 4.6 (45 min) and 6.0 (90 min) times, respectively. According to Table 4, there was no discernible relationship between cortisol levels and FTP scores.

3.3 Stress tests

3.3.1 Assessments of cortisol

Table 3 shows the plasma cortisol concentrations of each cat tested at different time points throughout the stress test: 90, 95, 100, 105, 115, and 180. The concentration of cortisol increased by 3.4 times, peaked at 15 minutes after the stressor (t = 105), and then started to decline gradually. By 90 minutes after the stressor, the concentrations had practically reverted to baseline. Cortisol concentrations at t = 90 and FTP+ scores were weakly correlated (rs = 0.70, P = 0.047; Table 4). Table 4 shows that there was a statistically significant relationship between the ACTH-induced cortisol concentrations at 45 minutes after administration and the stress test-induced cortisol concentrations at 15 minutes post-stressor (t = 105). In addition, the cortisol concentrations at t=105, t=115, and t= 180 were significantly correlated with one another (Table 4).

3.3.2 Assessments of behavior

Table 5 displays the frequency of behaviours that were observed both before and after the stressor exposure. In the experiments, the most common behaviours were resting and avoiding obstacles. These percentages were 37.5% and 29.4% before the spray bath, and 41.0% and 27.7% after the bath, respectively (median values; n = 8; Table 5). Hence, Fig. 2 shows the results of an analysis of the amount of time spent resting and engaging in avoidance behaviour throughout each measurement period. A negative correlation (rs = −0.55, P = 0.02) was found between stopping to rest and engaging in avoidance activity. Infrequent instances of aggressive behaviour were noted during the trial. Table 5 shows that cats' grooming habit increased significantly following the stressor (p<0.05). No other conduct differed significantly between the pre- and post-stressor periods (Table 5). Except for the three-minute spray bath, no behaviour was linked to stress-related cortisol levels (data not shown). During the 3-minute spray treatment, the animal spent an average of 7±3 seconds moving about and 19±7 seconds making noises (mean ± SEM; n = 8). Table 4 shows that there is a highly significant negative connection (rs = −0.90, p<0.001) between the amount of time spent vocalising and locomotor behaviour.

Frequencies of behaviour before and after a stressful event were recorded during the stress test. For "other behaviour" prior to and after to the stressor, the median values were 3.7% and 7.5%, respectively; these results were omitted from the study.

Table 5: The stress test frequency data collected before and after the stressful event. For "other behaviour" prior to and after to the stressor, the median values were 3.7% and 7.5%, respectively; these results were omitted from the study.

Behaviors	After stressful event (%)	Before stressful event (%)	P value
Contact attention	43.3	9.4	NS
Grooming	17.1	11.2	0.025
Resting/sleeping	7.3	34.1	NS
Eating treats	9	10.4	NS
Avoidance behavior	3.1	7.5	NS
Investigatory	6.2	14.8	NS
Aggressive behavior	41.3	24.2	NS

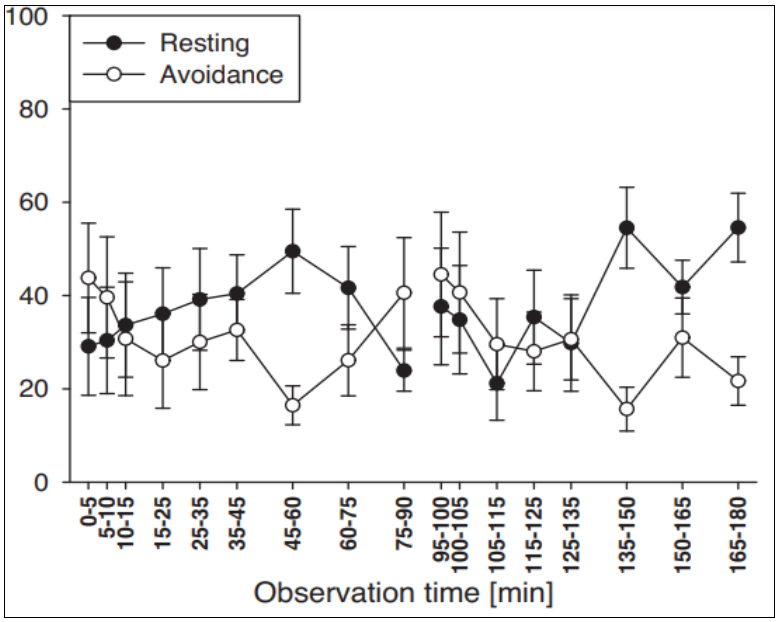

Fig 2: Resting and avoidance behavior frequencies during the anxiety test.

Video recordings were used for the purpose of behavior analysis. During the spray bath, no analysis was conducted on resting or avoidance behavior.

The plasma cortisol concentrations was significantly inversely correlated with the time spent moving (rs = −0.74, $p<0.05$) at t = 100/105, and strongly positively correlated with the time spent vocalising (rs = 0.93, $p<0.001$) at t = 100. It was also shown that the plasma cortisol concentration 45 minutes after ACTH administration was substantially linked with the duration of vocalisation (rs = 0.83, $p<0.001$) and movement (rs = −0.74, $p<0.05$) during the stress test.

4. Discussions

4.1 An adrenocorticotropic hormone (ACTH) and spray baths reaction

Peak plasma cortisol concentrations were observed during ACTH activation and the stress test. It follows that any and all cats could have participated in this study. Due to the high association between plasma levels of cortisol in the ACTH activation test (T=45) and the stress test (T=105), it appears that individual circumstances impacted the cortisol spike, whether it was caused by the stressor or ACTH. The time of day may have played an effect, given that both tests were conducted simultaneously at 12:30 h. For a long time, it was thought that the pituitary hormone ACTH was the only controller of glucocorticoid synthesis and release (Mormède *et al*., 2007; Ehrhart-Bornstein and Bornstein, 2008) [32, 12], even though the hormonal reaction to stress is a combination of the activated HPA axis and the sympathetic adrenomedullary systems. The interplay between the adrenocortical and adrenomedullary tissues appears to have a substantial impact on the stress response thus far (Ehrhart-Bornstein and Bornstein, 2008) [12]. The ACTH stimulation test, however, should have included sympathetic adrenomedullary system stimulation as a minimal factor.

The short and mild stressor "spray bath" was likely effective because the cortisol levels assessed at 105, 115, and 180 minutes into the stress test were highly correlated with one another.

Potent enough to influence cortisol levels for at least 90 minutes, therefore changing the patterns of adrenocortical secretion. The spray bath was deemed a "mild" stressor in this study since it did not produce any noticeable increases in plasma cortisol levels over an extended period of time. While ACTH stimulation led to a steady rise in plasma cortisol levels that were noticeably higher than baseline concentrations after 90 minutes, stress testing revealed that cortisol levels peaked within 15 minutes, fell to levels that were statistically indistinguishable from baseline concentration after 90 minutes. Rand *et al*. investigated the impact of a 5-minute spray bath on behaviour and cortisol levels (2002). After 30 minutes of exposure to the stressor, their results demonstrated that cortisol levels peaked and multiplied fivefold. Alternatively, cortisol levels remained elevated until the last blood sample was collected 100 minutes following the stressor. In our investigation, cortisol levels surged 3.4-fold, reached a peak at 15 minutes post-exposure, then decreased dramatically, and were nearly equal to pre-stressor values at 90 minutes post-exposure. According to Mormède *et al*. (2007) [32], the physiological response to acute stressor exposure, which led to both temporary and permanent alterations in blood cortisol concentrations, may be attributed to the stressor's intensity, or the duration of the spray bath. It is possible that the blood collection method, positive handling, and sufficient socialisation to humans were responsible for the quick decrease in post-stress cortisol values in this study. Potentially reducing the HPA axis's subsequent physiological stress reactions, these components were also present. Cahalane *et al*. (2007) [6], Morrison *et al*. (2007) [30], White (2002) [51], and Henry *et al*. (2002) [16] all document the successful use of vascular access ports (VAP) in cats and other companion animals. Venous access perfusion (VAP) enables researchers to collect blood samples from animals without causing them any discomfort. This technique allows for several plasma harvests with minimal restriction.

4.2 The connection between FTP and the way the adrenal glands react

There was no discernible difference in FTP scores between cats (Table 3). This might be because all of the cats were identical in appearance, age, and upbringing. They were also used to being handled in the same way and to the laboratory environment. Twenty female cats, each aged 10 months, were investigated four times over the course of eight months in a study conducted by Siegford *et al*. (2003) [44]. Over the course

of four measurements, the cats' FTP+ scores ranged from 5 to 22.8 and their FTP− scores from 0.75 to 5.75. The results showed that adult cats' feline temperament profile scores were consistent throughout the trial and agreed with ethological observations of the cats' human interactions. Table 4 shows that there was a high significant negative correlations between FTP (−) and FTP (+), suggesting that this test is a reliable way to distinguish between socialised and nonsocialised cats when it comes to humans. Veterinarians use it to estimate the baseline temperament of cats during clinical examinations, which helps with diagnosis and successful management, as well as for selection of cats in various settings like animal shelters and laboratories. Unfortunately, the FTP test failed to foretell the stress loading or reactivity in cats throughout our trial.

4.3 Connection between adrenocortical reactions and temperament

According to Koolhaas *et al*. (1999) [22], the way our bodies and minds respond to stress depends on its quality. Our three-minute spray treatment served primarily as a physical stressor, but the spatial confinement experienced by the cat within the cage added an additional psychological dimension. Consequently, the stressor in question needs to be capable of triggering both physiological and behavioural responses. Unfortunately, the experimental setting severely constrained the behavioural reactions. A cat was held on the lap of the test subject without active restraint for the stress test, which included the spray bath and its subsequent procedures. Since this was not a "normal condition," the cats could not act in the same way. Therefore, it is not possible to draw meaningful comparisons between the behaviour observed here and other experiments. The second most common behaviour, avoidance, was equally negatively correlated with resting behaviour and was recorded 29% of the time. In the stress test, the cats showed signs of avoidance behaviour, such as trying to escape or displaying a fear reaction, although their fear responses seemed to be relatively modest. The negative link between avoidance and resting behaviour is a consequence of the fact that time spent avoiding something may typically indicate more active behaviour than aversion, which is associated to fear. The fact that the cats' avoidance behaviour was somewhat reduced following stressor exposure suggests that they probably became used to the experimental settings. Between the hours of 8:00 and 16:00, Podberscek *et al*. (1991) [34] noted that eight cats in a laboratory setting engaged in maintenance behaviours (i.e., sitting, drinking, eating, and eliminating) for 36% of the time, comfort behaviours for 30%, and locomotory behaviours for 24.5%. Between the hours of 8:00 and 9:00 a.m., the study found that participants were most active, with high rates of walking, leaping, eating, drinking, scratching, rubbing, and defecating. During the less busy hours of 11:00 to 14:00 h, we conducted our experiment. No notable changes in behaviour were noted between the pre- and post-stressor periods, with the exception of grooming behaviour (Table 5). According to Eckstein and Hart (2000) [11], cats spend around four percent of their time grooming. Grooming has multiple functions, including keeping the skin clean and free of parasites, preserving the pelage's insulating properties, and regulating body temperature. Research by van den Bos (1998) [47] found that cats in a stable colony will frequently engage in autogrooming, or licking their own fur, immediately following fights. Acute stress response in social and non-social situations in cats may be indicated by autogrooming. The cats in this study may have been trying to alleviate some tension by increasing their autogrooming after the 3-minute spray bath, or they may have just been trying to maintain a clean, dry coat and skin. Our study found a favourable correlation between vocalisation time during the spray bath and cortisol concentrations in both the stress test (t = 100) and the ACTH stimulations test (t = 45). Animals' vocalisation reactions to different types of stress can tell us a lot about their mental health (Rushen, 2000) [41]. Several investigations have sought to gauge animals' reactions to stress by observing their vocalisations and other forms of communication (Beerda *et al*., 1998; Bristow and Holmes, 2007; Greiveldinger *et al*., 2007) [27, 19, 4]. According to Johnson *et al*. (1994) [18], pigs were shown to exhibit vocalization in addition to the release of ACTH and cortisol when injected with corticotropin-releasing hormone (CRH), which stimulated the HPA axis. Opposite to this, Beerda *et al*. (1998) [2] did not find any evidence linking increased vocalisation to acute stress in dogs. Contrarily, Bristow and Holmes (2007) [4] found that stressed-out cattle with elevated cortisol levels vocalised more, which is consistent with our findings in cats. In order to deal with temporary dangers, animals have developed coping mechanisms (Moberg, 2000) [9]. Because the nature of the stressor determines the strength of the association, conclusive evidence linking HPA axis activity to coping mechanisms is not necessarily present. Additionally, coping style may not be related to emotionality, and the HPA axis may be more associated with emotional reactivity (Koolhaas *et al*., 2007) [23]. We found that cats exhibited different coping styles during stressor exposure. Cats that walked around more during the spray bath and exhibited more vocalisations during stressor exposure may have a more active SAM system. On the other hand, cats that sounded more were more likely to have a reactive coping style, which is associated with a high plasma cortisol response and high HPA axis reactivity (Koolhaas *et al*., 1999) [22].

5. Conclusion

Finally, our data show that cats used vocalisations and movement as distinct coping mechanisms with the stressor spray bath. Vocalisations during stressful settings is indicative of HPA axis activation, according to a correlation between vocalisations and plasma cortisol levels. Behavioral measures as a short-term stress indicator appear to have limited information based on the slight differences in behavior before and after the spray bath, at least in laboratory conditions including cats. Under these circumstances, the FTP test failed to foretell the behavioral and adrenocortical reactions in anxious cats.

6. Conflict of Interest

Not available

7. Financial Support

Not available

8. References

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