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CHRONIC STRESS IN CATS: AND... WHAT CAN CORTISOL IN THE CLAWS AND FUR TELL US?



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OUTLINE FOR TODAY

- ✓ The case of Martin and his problems
- ✓ What we know or don't know about cat stress, needs, welfare
- ✓ Emotions and behavior in cats: fear and cognitive bias
- ✓ Stress response: acute, distress, chronic stress
- ✓ Chronic stress in cats and what we know
- ✓ Martin
- ✓ Measuring stress in cats and other species
- ✓ Study: Objectives, hypotheses; Methods; Results; Discussion
- ✓ Martin



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CASE: MARTIN



🔥 6yo, 4.9 kg, MN grey DSH

🔥 "biennial" exam for "shots"



"Stabby cat"
<https://www.thesoccompany.com>

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MARTIN DECLINES SERVICES TODAY

- 📌 Check history
- 📌 Owner expectations

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HAPPY CAT PACK!

- 📌 Gabapentin
- 📌 Instructions
- 📌 Squeeze treats
- 📌 Feliway wipes

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MARTIN RETURNS NEXT WEEK...

- 📌 PE: dander, somewhat unkempt, otherwise unremarkable
- 📌 CBC, chem w/ T4, U/A all WNL, urine culture negative

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MARTIN'S BEHAVIOR...

- 🔥 Staring at, chasing, biting another cat (Bart) in home
- 🔥 Vertical urine spraying (backed up to wall, quivering tail)



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MARTIN'S HISTORY: IMPORTANT!

- 🔥 Lives with 1 owner; 3 other adult MN cats (1 is Bart)
- 🐾 Martin (patient) adopted as kitten 6 yrs ago
- 🐾 Bart, 4yo MN DSH, adopted 1 year ago
- 🐾 Martin & Bart intro bad from outset: Martin stared, stiffened, growled, hissed, piloerection, chase, sprayed
- 🐾 Other 2 cats bonded with allogrooming, sleeping together, to both Martin & Bart
- 🐾 Martin hides from visitors; scared of loud noises
- 🐾 Martin pacing for past 6 months and spending less time with owner

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IS MARTIN EXPERIENCING CHRONIC STRESS?

- 🔥 Why does that matter?
- 🔥 How would you know if he's experiencing chronic stress?
- 🔥 Why does it matter if you know?

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WHAT DO "WE" KNOW ABOUT CAT BEHAVIOR, NEEDS, WELFARE → CHRONIC STRESS?

- Grigg and Kogan 2019: "Owners' attitudes, knowledge, and care practices: Exploring the implications for domestic cat behavior and welfare in the home."
- Da Graca Pereira et al 2014: "Comparison of interpretation of cat's behavioral needs between veterinarians, veterinary nurses, and cat owners"

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WE ARE ALL DEFICIENT

Let's LEARN!



RIP: "Cabo"

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EMO...

Emotions: responses to specific, personally salient events important to **INDIVIDUAL** (Bennett et al 2017)

A primary **function** of emotional responses is to mobilize in response to unexpected change and so they involve relatively innate, species-typical behavioral and physiological responses (Fox 2008).

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FACIAL CORRELATES OF EMOTIONAL BEHAVIOR

Bennett et al 2017: Facial correlates of emotional behaviour in the domestic cat (*Felis catus*)

- Used “CatFACS” to associate feline facial actions (FFA)/ expressions with emotional state

www.catfacs.com

What CatFACS is:

The Cat Facial Action Coding System (CatFACS) is a scientific observational tool for identifying and coding facial movements in cats. The system is based on the facial anatomy of horses and has been adapted from the original FACS system used for humans created by Ekman and Friesen (1978). The CatFACS manual details how to use the system and code the facial movements of cats objectively. The manual and certification is freely available (see below).

More info regarding the development of the FACS system can be found here:
www.catfacs.com/development-of-catfacs
www.catfacs.com/development-of-catfacs/
 (Date 10/20/2018)

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FEAR FACIAL CORRELATES

Bennett et al 2017: Facial correlates of emotional behaviour in the domestic cat (*Felis catus*)

- FEAR → frustration: ears flattening, ears **rotating**, nose lick
- Freezing, ventral flat posture, hiding and retreating
- Clinic/hospital or shelter: tend to **freeze** before attempting to escape or hide (Kry and Casey 2007)
- blinking/half blink (*NOT to be confused with “slow blink”*)

IMPORTANT POINT: Cat in a little “loaf” in the clinic, is most likely a **TERRIFIED** cat

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FELINE EMOTIONAL ETHOGRAM

Nicholson and O’Carroll 2021: Development of an ethogram/guide for identifying feline emotions: a new approach to feline interactions and welfare

EMOTION	BODY LANGUAGE				ACTIONS	RISK OF HANDLER INJURY	RISK OF WELFARE ISSUE
	EYES	EARS	TAIL	BODY			
Fear	Wide open eyes [25, 30] with round dilated pupils [25] ^a Blinking or half blinking [30] Or eyes tightly shut [31] or avoidance of eye contact [33, 36, 39]	Flattened [24] to the side [33, 35, 36] or back [32, 35, 36, 38] Ear pinnae are not visible [35]	Tucked under the body or wrapped around it [32, 36, 39]	Piloerection [26, 30] Tense muscles [32] Crouching [32, 38, 39] Lowered head [26, 30] Standing with arched back [35] Left head turn in mild fear [38]	Vigilance [39] Startle [39] Trembling [32, 39] Freezing [32, 38, 39] Hiding [32, 38, 39] Fleeing/avoidance [32, 38, 39] Grooming [32] No maintenance behaviours (eating, drinking, elimination/sleep) [39]	MODERATE	HIGH

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From: Nicholson and O’Carroll 2021

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NEGATIVE COGNITIVE BIAS

- Individuals with a negative state of mind are more likely to attend to aversive stimuli, recall more negative memories, and judge ambiguous information or uncertain situations as more negative than individuals with a more positive outlook and experiences
- Animals that are already experiencing negative emotions, such as fear, may be more likely to interpret an ambiguous stimulus as a threat rather than a signal of something neutral or positive

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AND WHAT ARE NEGATIVE EMOTIONS ASSOCIATED WITH?...

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LET'S TALK ABOUT...

STRESS

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STRESS

“... the biological responses an animal exhibits in an attempt to cope with a threat to its homeostasis.”
(Carstens and Moberg 2000)

“... normal and highly adaptive mechanism...”

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THE STRESS RESPONSE

- 🔥 Autonomic nervous system
 - 🔥 sympathetic
 - 🔥 parasympathetic
- 🔥 Hypothalamic-pituitary-adrenal (HPA) axis

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STRESS → DISTRESS → CHRONIC STRESS

When the stressor is **severe**, of **long duration**, or characterized by the **cumulative** effects of several stressors, then the total **biological cost of the stress response** may require the diversion of resources from other biological activities, disrupting other biological functions that are critical to the animal's well-being. When these functions are disrupted, the animal enters into a **prepathological state**, rendering it vulnerable to the development of pathologies. During this time, when normal function is disrupted and the possibility for pathology exists, the animal experiences distress and its **welfare is threatened**.

Carstens and Moberg 2000

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CHRONIC STRESS

Complex relationship:
+ individual, coping, environment, genetic

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CHRONIC STRESS IN CATS: WHAT WE DO KNOW

Behavior	Health
<ul style="list-style-type: none"> 🚩 Inhibition of behaviors: eating, grooming, elimination, exploration, play 🚩 Hiding, fighting, scratching, extreme vigilance 🚩 Disrupt environment 🚩 House soiling (physical illness → cycle) 🚩 Over grooming, under grooming, self-mutilation 🚩 Inappetance 	<ul style="list-style-type: none"> 🚩 Immune dysfunction 🚩 GI: vomit, diarrhea 🚩 FHV, URTD, idiopathic cystitis, inappetence → hepatic lipidosis 🚩 Inappetance → malnourishment → immune compromise

Poor welfare and Quality of Life (QoL)



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BACK TO MARTIN....

What are Martin's behavioral diagnoses?

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MARTIN DIAGNOSES

- 🔥 Intercat conflict with territorial aggression
- 🔥 Urine marking secondary to anxiety
- 🔥 Generalized anxiety disorder

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IS MARTIN EXPERIENCING/SUFFERING FROM CHRONIC STRESS?

- 🔥 We know it matters, right?
- 🔥 How do we know if he is or isn't experiencing chronic stress? Are there objective measures?

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STRESS MEASURES IN CAT

“Considering the popularity of the domestic cat as a pet, it is perhaps surprising that relatively little attention has been paid to validating measures of its welfare, by comparison with the amount of published material available for farm animals.”

– Casey and Bradshaw

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MEASURING STRESS IN CATS: WHERE ARE WE NOW?
BEHAVIOR ASSESSMENTS

Observation-based behavioral assessments of stress and poor welfare of cats

- McCune 1994: Caged cats: avoiding problems and providing solutions
- Kessler and Turner 1997: Stress and adaptation of cats housed singly, in pairs and in groups in boarding catteries
- Kessler and Turner 1999: Effects of density and cage size on stress in domestic cats housed in animal shelters and boarding catteries
- McCobb et al. 2005: Assessment of stress levels among cats in four animal shelters, JAVMA
- Iki et al. 2011: Relationships between scores of the feline temperament profile and behavioural and adrenocortical responses to a mild stressor in cats
- Moore and Bain 2013: Evaluation of the addition of in-cage hiding structures and toys and timing of administration of behavioral assessments with newly relinquished shelter cats
- Stella et al. 2014: Environmental factors that affect the behavior and welfare of domestic cats housed in cages
- Ellis et al. 2014: Behavioural and faecal glucocorticoid metabolite responses of single caging in six cats over 30 days
- Finka et al 2014: A critically appraised topic (CAT) to compare the effects of single and multi-cat housing on physiological and behavioural measures of stress in domestic cats in confined environments
- Weiss et al. 2015: Modification of the feline-ality assessment and the ability to predict adopted cats' behaviors in their new homes
- Duffy et al 2017: Development and evaluation of the Fe-BARQ: A new survey instrument for measuring behavior in domestic cats
- Rehnberg et al 2015: The effects of social interaction and environmental enrichment on the space use, behavior and stress of owned housecats facing a novel environment

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MEASURING STRESS IN CATS: WHERE ARE WE NOW

Arhant et al 2015: Assessment of behavior and **physical** condition of shelter cats as animal-based indicators of welfare

Gilhofer et al 2019 "Welfare of feral cats and potential influencing factors."

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MEASURING(?) STRESS IN CATS: CORTISOL?

<p>Blood</p> <ul style="list-style-type: none"> Acute, immediate changes w/in MINUTES; 1-timepoint; not systemic exposure Variation: HUGE = Daily, pulsatile, individual, activity, RESTRAINT, exercise, age, sex, procedure itself → STRESS! Saliva: as per blood 	<p>Urine</p> <ul style="list-style-type: none"> 4-8 hours prior to collection Bacterial degradation Cats secrete little cortisol in urine <p>Feces</p> <ul style="list-style-type: none"> Within past 24 hours Conjugation in liver, passage thru GIT, bacterial breakdown, digesta Varies: based on which part of feces, food type, storage after
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100% NOT chronic stress measures!

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CHRONIC STRESS MEASURES: HAIR CORTISOL?

- Slow growth rate
- Minimum length of time depends on rate of regrowth
- Is cortisol systemic/adrenocortical or local/derm origin

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CHRONIC STRESS MEASURES: HAIR CORTISOL

- Davenport 2006 – macaques (with saliva and stressful conditions)
- Validated for dogs
 - Bennett and Hayssen 2010
 - Bryan et al 2013
 - Veronesi et al 2015
 - Mack and Fokidis 2017
 - Grigg et al 2017
 - Roth et al 2016
 - Ouschan et al 2013
 - Siniscalchi et al 2013
- Validated in cats in 1 study; used in 2 others
 - Accorsi 2008 –
 - Finkler Terkel 2010
 - Galuppi 2013
- Other species

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MEASURING STRESS IN CATS: HAIR CORTISOL

- Accumulation, non-invasive: hair/fur (Accorsi et al 2008; Galuppi et al 2013; Finkler & Terkel 2010, 2015)
- Growth rate DIFFERS primary vs secondary (Baker 1974; Hendriks et al 1997)
- Does location matter? (lynx Tervissen et al 2013; bears Macbeth et al 2010; dogs Roth et al 2016; dogs Bennett and Hayssen 2010; vs dogs Bryan et al 2013)
- Source?
- Assay: RIA in cats



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CHRONIC STRESS MEASURES: NAIL CORTISOL?

- 🔥 Dogs
 - 🔥 Veronesi et al 2015:
 - 🔥 Mack and Fokidis 2017
- 🔥 Cows and calves (Comin et al 2014)
- 🔥 Chameleons (Matas et al 2016)
- 🔥 Less variable?
- 🔥 Assays: EIA, RIA

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CAT NAILS!

- 🔥 Cats
 - 🔥 No studies to date
 - 🔥 Nail growth rate unverified
- 🔥 Keratinized epithelium
- 🔥 In center: corium with blood supply and nerve – cortisol passively accumulates

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SUMMARY: CORTISOL MEASURES IN CATS

Sample	Invasive	Cortisol affected by sampling technique	Period represented	Other Cons
Blood	yes	Yes; sharp increases (10x) within 3-5 minutes; (handling stress)	Single-point: minutes	Affected by CBG; can't measure free unbound biologically active fraction; inconsistent; pulsatile; daily fluctuations
Saliva	Potentially in cats	Possibly in cats; handling stress responsive within 20-30 minutes	Single-point: minutes	Daily fluctuations; need enough saliva
Urine	Dependent on collection system	unlikely	Approximately 4-8 hours	Microbial degradation; less than 15% GC metabolites excreted in urine; storage control issues
Feces	no	no	Most within 24-48 hours	Dietary changes; microbial degradation; not evenly distributed; storage control issues
Hair	no	no	Weeks, months	Coat color; seasonal molting
Nail	no	no	Weeks, months	Unknown growth rates in cats

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IMAGINE...

If we could objectively measure whether Martin is experiencing chronic stress...

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OBJECTIVES AND HYPOTHESES

Objectives:

1. Evaluate use of EIA to quantify hair cortisol concentrations (HCC) and nail cortisol concentrations (NCC) in cats
2. Determine whether either HCC and/or NCC might be used to evaluate chronic stress in cats

Hypotheses:

1. HCC from different body locations will be similar
2. HCC will be positively associated with NCC
3. Behavioral and physical factors potentially indicative of chronic stress in cats will be associated with HCC or NCC
4. NCC will be more consistent than HCC

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METHODS: CATS

Owned or community adult cats (n=52)

3 recruitment stages:

1. Stressed (assay validation) (n=3)
2. Healthy; staff or S/N (n=16)
3. Variety of issues (n=33)

Exclusion: nail trims; glucocorticoids

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METHODS: SAMPLES

- 🔥 2x2 cm hair patches
- 🔥 varied locations (n=19)
- 🔥 LS only (n=29)
- 🔥 nails (n=48)

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METHODS: QUESTIONNAIRES AND INFO

- 🔥 Medical (1-2 mo) owner-report
- 🔥 Daily lifestyle (1-2 mo) owner-report
(Mariti et al 2017; Contreras et al 2018; Duffy et al 2017)
- 🔥 Environment
- 🔥 Activities and Behaviors
- 🔥 Physical
- 🔥 Personality characteristics

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METHODS: CORTISOL EXTRACTION AND ASSAY

- 🔥 Commercial laboratory (University of Colorado BIEL)
- 🔥 Commercial Enzyme Immunoassay kit (Salimetrics LLC)
 - 🔥 Procedure: Davenport et al 2006; Lafferty et al 2015; D'Anna-Hernandez et al 2011; Hoffman et al 2017
- 🔥 Laboratory validation of assay to quantitate cortisol in feline hair and nails
- 🔥 pg/mg

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ANALYSES

- CCC and Bland-Altman: hair cortisol and body areas
- Spearman's rank correlation: HCC (LS) and NCC
- Correlational matrices and regression models

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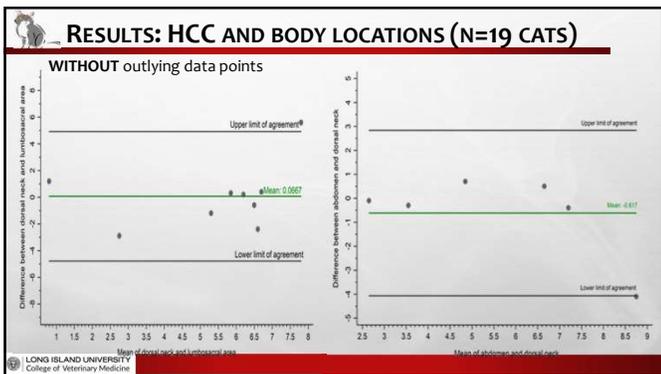
RESULTS: CATS AND CHARACTERISTICS (N=48 CATS)

- IM: 10.4%
- NM: 47.9%
- IF: 14.6%
- SF: 27.1%
- Age: median 11.8yo (1.5-20.0)
- 20.8% community cats



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RESULTS: HCC AND BODY LOCATIONS (N=19 CATS)

TABLE 1 Summary statistics for hair cortisol concentrations (pg/mg) by body location from which hair samples were obtained from the first 19 cats

Body location	Number of cats	Median (range)
Abdomen	6	5.5 (2.7-10.8)
Dorsal neck	17	6.2 (1.3-52.5)
Ventral neck	5	17.7 (3.0-224.1)
Lumbosacral	10	6.3 (0.2-251.5)

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RESULTS: HCC (LS) (N=39)

Median HCC 4.2 pg/mg (0.2-251.5)

P = .003; R2 = 0.366;
adjusted R2 = 0.315
Litterbox issues (P=.02)
Groomed, soft fur (P=.02)

$r_s = 0.70$; P < .001 (HCC: NCC)

Variable	Level	Number	P value	
Categorical variables	Chronic illness	No	16	.08
		Yes	23	
	Dander and mats	No	17	.03 ^b
		Yes	18	
	Declaw status	No	34	.02 ^b
		Yes	5	
	Feral or community cat	No	30	.14
		Yes	9	
	Friendly, engaging demeanor	No	9	.01 ^b
		Yes	21	
	High perches available	No	10	.05
		Yes	22	
	Home is quiet	No	6	.08
		Yes	24	
Litterbox issues*	No	18	.02 ^b	
	Yes	10		
Plays	Never	4	.09	
	Sometimes	5		
	Always	21		
	Soft and groomed hair coat*	No	10	.06
	Yes	28		
Continuous variables	Number of dogs	0-4	30	.02 ^b
	Stress axis (owner assessment)	0-80	30	.03 ^b

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RESULTS: HCC (LS) (N=39)

NOT associated with:

- Coat or sample color
- Hair sample length
- Neuter status
- Indoor vs outdoor
- Litterbox ratio
- # of cats in home

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RESULTS: NCC (LS) (N=48)

Median 0.6 pg/mg (0-10.3)

$P = <.001$; $R_2 = 0.379$;
adjusted $R_2 = 0.341$
Litterbox issues ($P=.001$)
Dander, mats ($P=.01$)

Variable	Level	Number	P value
Categorical variables			
Chronic stress	No	23	.001*
	Yes	25	
Dander and mats*	No	25	.001*
	Yes	19	
Declaw status	No	42	.02*
	Yes	6	
Fights with or hides at other cats	Never	19	.06
	Sometimes	11	
	Always	5	
Feline immunodeficiency virus (FIV) positive	No	25	.12
	Yes	7	
Friendly, engaging demeanor	No	14	.02*
	Yes	25	
Greets owner	No	5	.1
	Yes	22	
Home is quiet	No	30	.04*
	Yes	28	
Intact reproductive status	No	26	.02*
	Yes	12	
Litterbox issues*	No	24	.02*
	Yes	12	
Nails last trimmed	Never	12	.02*
	Greater than 1 mo	22	
	Between 2 and 4 wk	7	
Soft and groomed hair coat	No	11	.02*
	Yes	26	
Continuous variables			
Age (yr)	1.5-20.0	48	.001*
Number of dogs	0-4	27	.06
Stress index (owner assessment)	0-80	38	.12

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RESULTS: NCC (LS) (N=48)

NOT associated with:

- M/F
- Indoor vs outdoor
- Litterbox ratio
- # cats in household
- Sneezing



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DISCUSSION:

Hypotheses:

- ~~HCC from different body locations will be similar~~
- HCC will be positively associated with NCC
- Behavioral and physical factors potentially indicative of chronic stress in cats will be associated with HCC or NCC
 - YES: poor hair coat and house-soiling**
- NCC will be more consistent than HCC
 - YES**

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DISCUSSION

- Unkempt appearance (dander, matted fur vs soft fur)
- House-soiling
- NCC more consistent but lower than HCC
- HCC varied **WIDELY** within individual cats on SAME day



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DISCUSSION: HCC

- Variation: (Macbeth et al 2010; Carlitz et al 2015; Terwissen et al 2013; Roth et al 2016; Grigg et al 2017)
 - Growth rates
 - Guard vs undercoat
 - Location
 - Section
 - Models driven by a few cats
- Extrapolated time pd for cortisol accumulation: ~ 3 mo's?



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DISCUSSION: NCC

- Growth rates
 - Extrapolated time pd for cortisol accumulation: ~ 21-day period at least 1 month prior to trimming (publication pending)
- Extraction
- Front vs rear (pub pending)



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LIMITATIONS

- Small sample size
- Observational, no control group,
- Owner report and varied histories
- Timeframe of cortisol



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CONCLUSIONS AND FUTURE

- Reliability of single HCC measurement in cat is QUESTIONABLE
- NCC measurement might be a valuable, objective, mostly non-invasive diagnostic tool
- Ongoing studies/manuscripts: front vs rear, growth rates, repeatability studies
- Is cortisol accumulation in nails related to chronic stress? Or individual? Or something else?



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WHAT ABOUT MARTIN?



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MARTIN TREATMENT

- 🔥 Environmental management
- 🔥 Behavior modification
- 🔥 Psychopharmaceuticals
 - 👉 Fluoxetine (chronic)
 - 👉 Gabapentin (acute)
 - 👉 Pheromones

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MARTIN'S OUTCOME

- 🔥 3-month follow-up: marking significantly decreased
- 🔥 6-month follow-up: Martin & Bart allogrooming! – and only 1 urine marking incident

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WAS MARTIN SUFFERING FROM CHRONIC STRESS?

IS MARTIN STILL SUFFERING FROM CHRONIC STRESS?

- Wouldn't it be super cool if we had an objective measure to use, that could tell us if Martin was experiencing some level of chronic stress?
- Part of a MDB, add-on test, a PORTION of the overall picture and assessment of Martin's case

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THANK YOU
~
QUESTIONS?



elena.contreras@liu.edu  [#maxximosthecat_andfriends](https://twitter.com/maxximosthecat_andfriends)

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