



Puppies and kittens as models of impaired neurobehavioral development: Early blunted cortisol is associated with poor performance on social and cognitive tests



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Introduction:

Dogs and cats may be excellent non-induced, non-lesional models for human psychopathology. We studied behavioral, physiological, and epigenetic consequences of early-life stress in a population of 50 homeless rescue kittens and in a population of 36 owned, volunteered, puppies, all from the Atlantic Maritimes.

Methods:

Kittens (N=50) underwent 4 behavioral tests (open field, feather toy interaction, approach novel human, held by novel human) across cognitive domains 1, 3, & 4 (see dog domains below) at 8, 10, and 12 weeks of age. A 3 x 3 cm hair sample (hair cortisol concentration (HCC)) was obtained at 8 weeks and a whole blood sample (relative telomere length (RTL)) was obtained at ~10 weeks. Puppies (N=36) underwent cognitive tests at 3, 6, 9, and 12 months. At those same times clients completed a standardized, validated questionnaire (WDQ-Pet) about behavioral responses, rearing, husbandry, training, diet, etc.. Test results were analyzed with respect to outcome, time, distance, and scored behaviors from video-developed ethograms. A 3 x 3 hair sample (HCC) was obtained at 3 and 12 months; a whole blood sample (RTL) was obtained at 6 and 12 months. Here we concentrate on HCC and associations with time to approach and time held for kittens, and for puppies, HCC and scored outcomes of the noise test.

Canine cognitive/problem solving tests:

1. Approaching a novel human (*Domain 1: Social learning*)
2. Tracking a moving toy (*Domains 1 & 4: Social learning/Executive function*)
3. Responses to behavioral requests (sit, down, stay) when you can and cannot see the person's face (*Domains 1 & 4: Social learning/Executive function*)
4. Open field test (boxes, toys, balls) (*Domains 2 & 4: Spatial learning/Executive function*)
5. Searching for a toy the dog has seen hidden (Referential signaling: show) (*Domains 2 & 4: Spatial learning/Executive function*)
6. Searching for an unseen toy where location is indicated (Referential signaling: knock, point, flag) (*Domains 1, 2 & 4: Social/Spatial learning/Executive function*)
7. Noise test (*Domains 3 & 4: Spontaneous behavior/Executive function*)

Results:

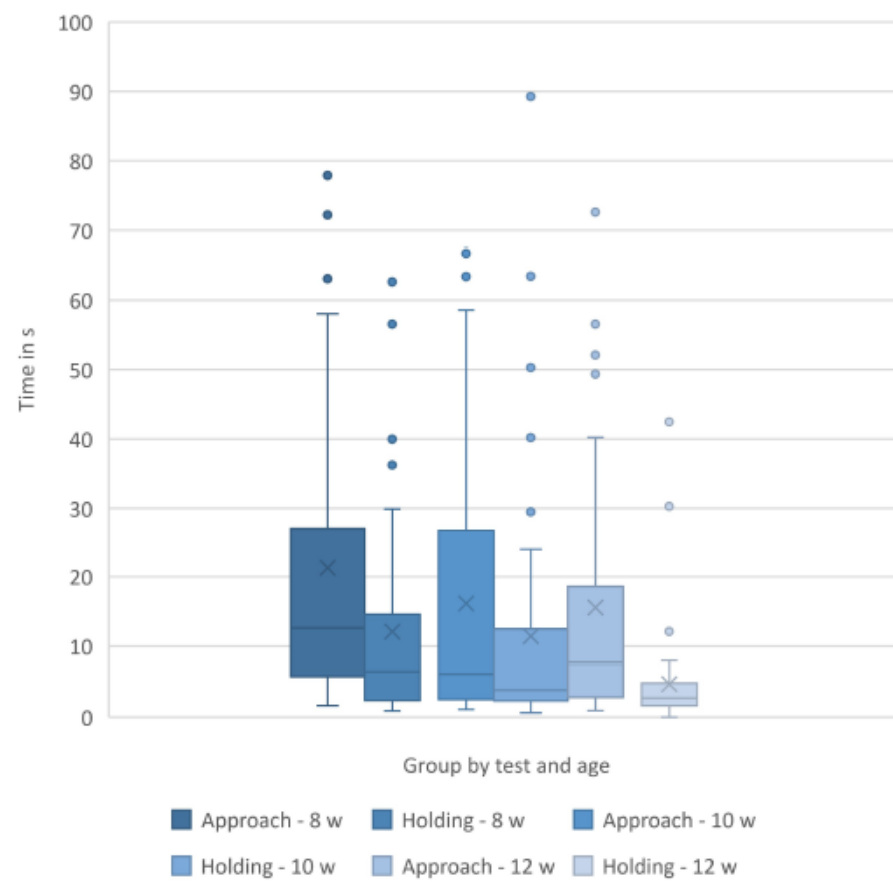
Kittens

Kittens had 120 s to approach a novel human and did so more quickly at 10 and 12 weeks (Friedman test, χ^2 ; $r = 7.54$ (2,39);

$p = 0.02$) than at 8 weeks, but there was no difference in approach time between 10 and 12 weeks.

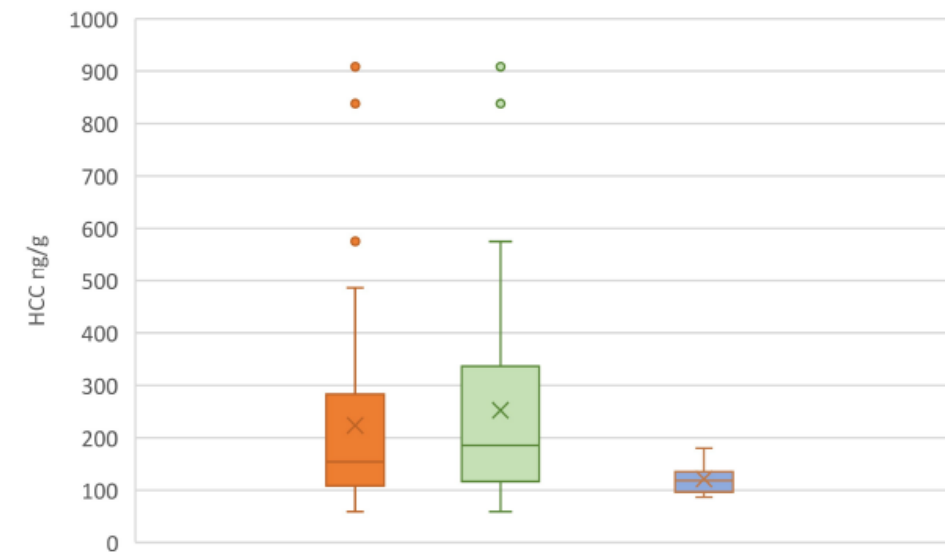
If kittens that *did not approach* were removed from the holding time data *across all weeks* (N = 11), the longest holding attempt significantly differed at each of the three ages tested and *decreased with age* (Friedman test, χ^2 ; $r = 7.54$ (2,39); $p = 0.02$).

If any kitten with an *extreme holding time* (> 120 s) at any week was removed from the data across all weeks (N=5), the longest holding attempt significantly differed at each of the 3 ages tested and *decreased with age* (ANOVA $F = (2,88) = 5.00$, $p = 0.009$).



The distribution of approach latencies and longest holding times for kittens who approached and did not have extreme (>120 s) holding times (N = 39 for approach times; N = 45 for holding times).

Note: For all graphs, the box and whisker plots show the means (x), the medians (lines), the box contains 50% of the scores, whiskers are 75% of indexed scores for each group.



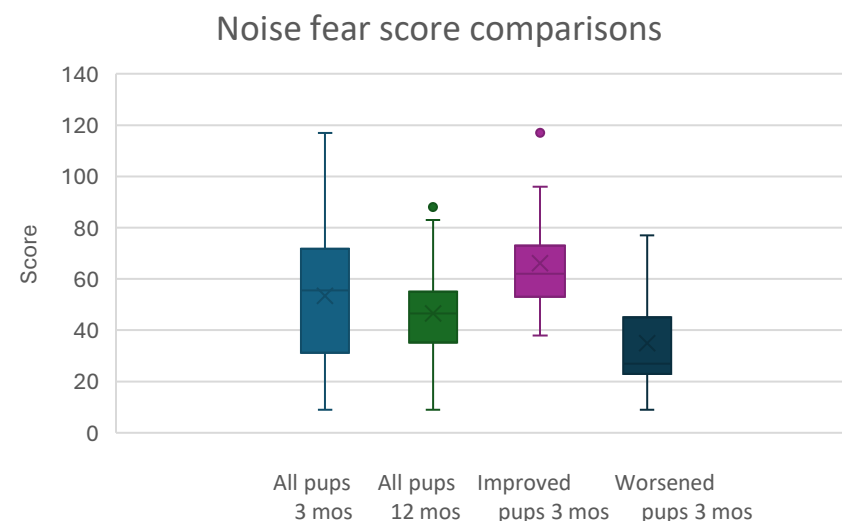
HCC across subgroups of kittens: Summary data for HCC across the entire group of kittens (orange), with kittens who did not approach removed (green), and for those of the kittens that did not approach on at least one occasion (plum).

Kittens who did not approach at least once over the three test periods had a significantly lower mean HCC than the rest of the kittens, while the group of kittens where everyone approached had significantly higher HCC (Mann-Whitney U; $p = 0.02$; z-score = 2.32; U = 115).

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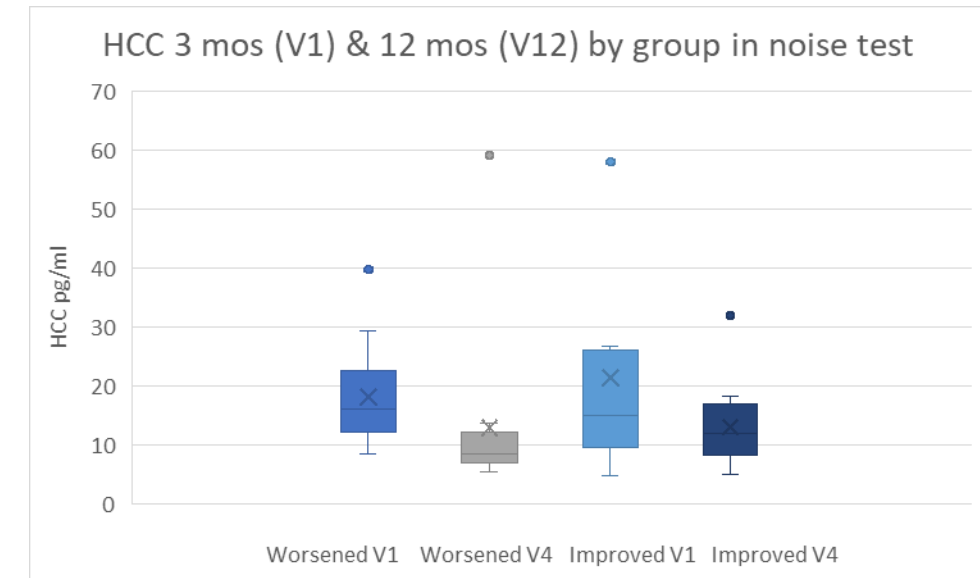
Puppies

From 3- to 12-months, 50% of our tested puppies became significantly more fearful in the noise test (ANOVA (1,35); $F=22.643$; $p=0.00003$), 42% in the referential signaling test (T-test: $t=2.252$; $p = 0.032$), 34% in the open-field test (T-test: $t=5.609$; $p < 0.00001$), and 33% in the approach test (T-test: $t = 2.252$; $p = 0.032$). Here, our focus is noise.



Pups that improved vs. worsened from 3 to 12 months differed in their fearful behavior scores on the noise test at 3 months (ANOVA (1,35); $F=22.643$; $p=0.00003$).

Dogs that worsened on tests from 3 to 12 months scored high at 12 months on all the WDQ-Pet questionnaire sections measuring relevant problematic responses. For the noise test discussed here, dogs that became *more fearful by 12 months* had significantly lower HCC than those that did not.



Comparison of HCC for pups that worsened from visit 1 to 4 on the noise test (Mann-Whitney U test; $U = 25$; $z=2.665$; $P<0.008$) and those that improved from visit 1 to 4 (T-test (1 tailed); $t=1.805$; $p=0.040$).

For pups below the mean fear score in the noise test at visit 1 (3 months), the relative risk of worsening on the test by visit 4 (12 months) is 1.32; the odds ratio of worsening is 1.67.

Conclusions:

Poor performance in repeated measures interaction tests with humans in 50 homeless kittens and on cognitive tests in 36 volunteered puppies from varied sources/locations correlated with extremely low (blunted) HCC at 8 weeks and 3 months of age, respectively, a pattern noted in lab rodent and human transgenerational studies. Almost half of the puppies were fearful of some noises by 12 months which was predicted by their test scores at 3 months.

Going forward, using the most informative of our tests, we are developing a citizen science project that includes genomics to further focus on effects of early life stress on diagnostic risk. An approach that improves assessment of risk/outcomes in companion animals may aid in similar approaches for humans.

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