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Independent Study 490A:
5 vs. 10-d of Handling; Which One is Better?

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Summary and Implications
The adoptability of an animal from a shelter largely depends upon its socialization and friendliness towards humans. For kittens, habituation and proper socialization is an important part of ensuring that the adult cat it will be able to interact properly with humans, thus reducing its chance of being relinquished in the future. In addition, kittens that have been relinquished or placed into a shelter are often subject to several stressors that may impact not only the well-being of the kitten but impair further socialization attempts. The objective of this study was to determine if there were behavioral differences between two handling regimes for kittens relinquished to the ARL-IA. This study was conducted at the Animal Rescue League of Iowa (ARL-IA), and involved 31 neonate kittens of mixed sex and breed, between 6 and 8 weeks of age. Treatment (5-d) five consecutive days of handling and treatment two (10-d) ten consecutive days of handling. During treatment kittens were exposed to several tests. The experimental unit was the kitten and a complete randomized experimental design was utilized. PROC GLIMMIX was used. A P-value of P < 0.05 was considered significant. There were differences (P < 0.05) between treatments for the amount of time that kittens were willing to let their rear paws be held. Kittens assigned to 5-d allowed both their rear paws to be held longer than those from the 10-d treatment. For all other handling measures there were no differences. In conclusion, handling kittens over 10-consecutive days indicated that kittens became less tolerant of having their rear paws held compared to a 5-d treatment and therefore there may be an optimal amount of handling before kittens begin to find this procedure more aversive.

Introduction
Kittens which are relinquished or placed into a shelter can be exposed to a barrage of novel or unfamiliar situations that may impinge on the individual kittens’ well-being. It may be possible to reduce and/or eliminate stressors that can act individually or in concert that ultimately affect the kitten whilst staying at the ARL-IA. Identified stressors may include other animals, people, facility design, and transportation to the shelter, changes in nutrition and the environment. Stressors that impinge upon a kitten may vary in time, intensity, mode, and degree of novelty. The kitten is developing mechanisms to deal with both acute and chronic stressors. The “additive stressor model” concept was proposed by Broom and Johnson in 1993. Using ARL-IA shelter as an example, if a kitten is subjected to multiple stressors within a short period of time, and time is not allowed for rest and recovery in between each subsequent stressor application, the kitten’s baseline physiological levels may rise and the kitten will take longer to recover. In the most severe of cases, the kitten may become fatigued, sick or even die.

The objective of this study was to determine if there were behavioral differences between two handling regimes for kittens relinquished to the ARL-IA.

Materials and Methods
The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee (1-11-7057-F). The experiment was conducted over March and April 2011.

Arrival: Upon arrival at the ARL-IA kittens were subjected to a health check performed by a vet tech, which included a check-up, administration of a dewormer, and vaccinations. All kittens were then allowed 3-d to acclimate to their new housing.

Animals, housing and feeding: This study was performed at the Animal Rescue League (ARL) of Iowa, located in Des Moines, IA. A total of 31 neonate kittens mixed sex and breed were observed. Neonate was defined as eyes and ear canals open. Kittens ranged between 6 and 8 wk of age and weighed between 680 and 970 grams. All behavior evaluations were conducted by two trained undergraduate research assistants. All kittens were brought in as strays and did not have a Queen. Kittens were kept as the litter that they were brought in as. In the cattery room there were 8 cages. Each cage measured 0.66 m wide x 1.2 m long x 0.8 m high. The cage had stainless steel wire meshing at the front. In each there as one water bowl and one feed bowl and kittens were provided bedding material. Kittens were observed at least three times a day by the ARL-IA staff.

Treatments: Two treatments were compared: 5-d (n = 14) vs. 10-d (n = 17) of consecutive kitten handling. Handling of kittens by other staff members was limited to spot clean during the trial time period.
Handling procedure: The day before testing began, the stomachs of the kittens were shaved and each kitten was identified by a number on their shaved stomach. On the day of testing one handler was responsible for handling of kittens regardless of treatment. Cell phones were turned off before entering the room and hands were washed. All clothing that the handlers wore had not been around other animals to reduce the issue of unintentional exposure to a sensory environment. One kitten at a time was removed from the cage, proceeded through the handling tests and was then replaced back into the cage with their littermates. The order of testing each day for the kittens was done in a randomized order but the order of tests was consistent (Table One). During the test no verbal or physical reinforcement/correction was directed towards the kitten by the handler.

Statistical Analysis: The experimental unit was the kitten and a complete randomized experimental design was utilized. PROC Univariate determined that the data was not normally distributed. PROC Glimmix (SAS) was used to analyze the data. The statistical model included the parameter of interest (handling measures from table one), treatment (5 vs. 10-d of consecutive handling) and kitten age was a linear covariate. The random statement was kitten nested within treatment. A P-value of $P \leq 0.05$ was considered significant and I-Link was performed to transform values for means and standard errors.

Results and Discussion
There were differences ($P < 0.05$) between treatments for the amount of time that kittens were willing to let their rear paws be held. Kittens assigned to 5-d allowed both their rear paws to be held longer than those from the 10-d treatment. For all other handling measures there were no differences (Table Two).

In conclusion, handling kittens over 10-consecutive days indicated that kittens became less tolerant of having their rear paws held compared to a 5-d treatment and therefore there may be an optimal amount of handling before kittens begin to find this procedure more aversive. This data could be further used in developing socialization and handling acclimatization programs for kittens in shelters, thereby increasing their adoptability and overall well-being, both in the present and in the future.

Acknowledgements
Special thanks to the felines and all the staff of the Animal Rescue League of Iowa.
### Table One: Handling Tests

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
<th>Recording levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Held to chest</td>
<td>Pick up kitten under belly. Bring kitten to upper chest. Rear legs placement on one hand, second hand support kitten body. Hold for 10 s.</td>
<td>1: Remains calm, purrs, moves in for interaction. 2: Struggles but struggling reduces for at least the last 5 s. Mild vocalization. 3: Tries to escape continuously, excessive vocalization, becomes fractious.</td>
</tr>
<tr>
<td>Repetitive stroke</td>
<td>Pick up kitten under belly. Place kitten onto test table with towel. Run hand from base of kitten’s neck to tip of tail. Repeat three times.</td>
<td>1: Remains calm, purrs, arches into petting, moves in for interaction. 2: Moves around on table, accepting of petting, does not leave table. 3: Tries to escape continuously, excessive vocalization, becomes fractious.</td>
</tr>
<tr>
<td>Ear check</td>
<td>Ear check: holding the kitten’s head to complete a visual inspection of one ear for 3 s.</td>
<td>1: Remains calm, purrs, body relaxed, accepts handling. 2: Tries to avoid handling, Mild vocalization, does not leave table, and accepts after first interaction. 3: Tries to escape, excessive vocalization, becomes fractious.</td>
</tr>
</tbody>
</table>

### Table Two: Behavioral measures for kittens handled 5 or 10 consecutive days at the ARL - Iowa

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
<th>5</th>
<th>10</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Held</td>
<td>Lift up kitten tip for 3 s.</td>
<td>1.22 ± 0.13</td>
<td>1.24 ± 0.08</td>
<td>0.90</td>
</tr>
<tr>
<td>Repetitive stroke</td>
<td>Scruff and restraint on the table and release.</td>
<td>1.10 ± 0.13</td>
<td>1.21 ± 0.08</td>
<td>0.50</td>
</tr>
<tr>
<td>Left ear</td>
<td></td>
<td>1.40 ± 0.21</td>
<td>1.36 ± 0.16</td>
<td>0.90</td>
</tr>
<tr>
<td>Right ear</td>
<td></td>
<td>1.11 ± 0.18</td>
<td>1.47 ± 0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>Right teeth</td>
<td></td>
<td>1.44 ± 0.20</td>
<td>1.30 ± 0.16</td>
<td>0.90</td>
</tr>
<tr>
<td>Left teeth</td>
<td></td>
<td>1.58 ± 0.21</td>
<td>1.72 ± 0.15</td>
<td>0.62</td>
</tr>
<tr>
<td>Scruff</td>
<td></td>
<td>1.05 ± 0.12</td>
<td>1.13 ± 0.08</td>
<td>0.59</td>
</tr>
<tr>
<td>Paw front right</td>
<td>Scruff and restraint on the table, held for 0.68 s and release.</td>
<td>7.13 ± 0.71</td>
<td>6.80 ± 0.35</td>
<td>0.70</td>
</tr>
<tr>
<td>Paw front left</td>
<td></td>
<td>7.60 ± 0.21</td>
<td>6.42 ± 0.33</td>
<td>0.54</td>
</tr>
<tr>
<td>Paw rear right</td>
<td></td>
<td>10.18 ± 0.98</td>
<td>5.76 ± 0.34</td>
<td>0.0003</td>
</tr>
<tr>
<td>Paw rear left</td>
<td></td>
<td>8.62 ± 0.80</td>
<td>6.52 ± 0.34</td>
<td>0.03</td>
</tr>
<tr>
<td>Righting</td>
<td></td>
<td>1.40 ± 0.14</td>
<td>1.30 ± 0.09</td>
<td>0.32</td>
</tr>
</tbody>
</table>
| Paw grasp        | Kitten is placed on the human’s lap, gently tip kitten back towards your body. Each paw is gently grasped by the human and held for 10 s (mimic trim/paw). Once paw is pulled back, test on that paw is concluded. | Length of time for paw to be pulled back/away.