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The effect of premature maternal separation on distress vocalizations and activity in kittens (*Felis catus*) during a brief nest separation

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Abstract

It is unknown how premature maternal separation affects the responses of kittens to potentially stressful events. In the United States, thousands of kittens are orphaned each year due to death of the queen, neglect, or accidental separation by humans. Neonatal mammals emit distress calls and increase locomotion when socially isolated, suggesting that being separated from the nest is a stressful event. Increased vocalization and activity of isolated neonates may aid maternal retrieval or relocation of the nest. In the current study, we assessed the effects of early maternal separation on later vocalizations and activity of 49 kittens (28 orphaned, 21 mother-reared; 23 female, 26 male) from 11 litters (5 mothered, 6 orphaned) during an open field test when the kittens were one and three weeks of age. We conducted a total of 79 trials.

Each kitten was placed individually in a 1-meter diameter pen away from the rest of the litter and/or mother for two minutes. The number of calls emitted and total activity (in seconds) were recorded for each kitten on every trial. We assessed the effects of age, sex, orphan status, and interactions between orphan status with sex and age on activity and vocalizations. Orphaned kittens were more active than mother-reared kittens at both times ($t(46) = 4.62, p < 0.001$), with an interaction between age and orphan status ($t(28) = -2.84, p = 0.008$). Orphaned kittens emitted more vocalizations at both times ($Z = 2.38, p = 0.018$), with an interaction between age and orphan status ($Z = -3.18, p = 0.001$).

Orphaned kittens showed increased activity and vocalizations in response to a brief nest separation compared to mother-reared kittens. This effect was still present after over two weeks of maternal separation, suggesting that maternal separation may lead to long-term changes in stress responses. Future research should explore if such effects of maternal separation are present in older kittens or adult cats.

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Keywords

maternal separation; neonatal development; stress responses; distress calls; domestic cats

Introduction

Neonatal mammals and some birds emit distress calls and increase locomotor activity almost immediately when isolated or separated from their nest (Lingle et al., 2012; Hudson et al., 2017). There are close neural connections within the brain between the vocal centers and the amygdala, which is highly related to the processing of emotions and resulting behavior (Rasia-Filho et al., 2000). Thus, vocalizations are believed to be closely related to an individual's emotional state, especially for negative states such as alarm, fear, or pain (Jürgens, 2009; Briefer, 2012). These distress calls share structural similarities across species and are likely highly conserved because they serve a protective function for neonates (Lingle et al., 2012). Such calls are usually emitted repeatedly until the neonate is retrieved by its mother (Christensson et al., 1995; Haskins, 1977). The repeated, high-pitched vocalizations of young animals frequently result in parental search and retrieval (Lingle et al., 2012).

Isolation may lead to other behavioral changes in neonatal animals. Many neonates will orient toward their nest if removed, and increased locomotion is thought to enhance the probability that the young will relocate their nest (Freeman and Rosenblatt, 1978). However, the interpretation of activity in relation to emotional state is not always clear; decreased locomotion has been attributed to increased fear or anxiety in many open field tests (Russell, 1973), but chronic, mild stress was related to increased activity in rats in an open arena (Grønli et al., 2005).

A previous study found consistent individual differences across three weekly test sessions of 33 neonatal mother-reared kittens, in terms of both the number of vocalizations they emitted and duration of activity exhibited during a three-minute nest separation (Hudson et al., 2015; Hudson et al., 2017). Kittens were placed in a 1-meter diameter arena in a separate area from their mother and littermates. Vocalizations and activity, defined as displacement of the body by moving all four paws, were recorded. Vocalizations were consistent across test sessions, such that kittens who exhibited a comparatively high number of vocalizations in the first session also did so in subsequent test sessions ($r = 0.49$). There was a correlation of duration of activity between sessions, but the effect was not as robust ($r = 0.17$; Hudson et al., 2015). In the current study, we further explored vocalizations and activity in neonatal kittens during a nest separation by adding a comparison group: orphaned kittens.

In the United States, thousands of kittens are orphaned each year due to death of the queen, neglect, or accidental separation by humans. Kittens are altricial at birth, so maternal separation early in life is a threat to survival. Orphaned neonatal kittens are one of the most vulnerable populations in animal shelters, and euthanasia is common due to shelters' limited resources and time to care for them (Kerr et al., 2018; Manning, 2014). Many rescue organizations are attempting to improve the standards of care by placing kittens in foster homes where they can be reared by humans. However, it is not fully understood to what

extent early maternal separation affects the behavior of orphaned kittens, including their responses to potentially stressful events.

Premature maternal separation occurs during the sensitive period of socialization for domestic cats, which is considered between two and seven weeks of age (Karsh & Turner, 1988). Handling by humans during the sensitive period, respective of maternal presence, improves later cat-human relationships (Bradshaw et al., 2012). Kittens normally spend significant amounts of time in physical contact with their mother and nursing in the first several weeks of their lives (Rheingold and Eckerman, 1971; Beaver, 2003). Maternal contact during this period is considered critical for the physical and behavioral well-being of kittens (Bradshaw et al., 2012). Contact with the mother decreases when kittens start weaning at approximately a month old but may continue into adulthood, especially in matrilineal colonies.

The goal of this study was to determine if the responses of orphaned kittens during a brief nest separation were different from those of mother-reared kittens. To test their responses, we used an established open field behavioral test at their first and third week of life. We separated kittens from their nest for two minutes and recorded the number of vocalizations and duration of activity during the separation.

There have been few studies of the effects of being orphaned on the stress responses of neonatal animals. Previous studies have focused on the effects of short-term isolation from the mother (e.g., 3 hours). It has been established that short-term maternal separation quickly results in increased distress calls by the young of various mammalian species (Hudson et al., 2015). For example, observational studies of orphaned chimps suggest they display more anxious behavior compared to mother-reared counterparts (Botero et al., 2013). The continual absence of the mother may lead to heightened stress responses, and this effect may be more pronounced when the separation is relatively recent. Therefore, we hypothesized that orphaned kittens would emit more vocalizations and be more active than mother-reared kittens during the test at 1-week of age.

Longer-term separation from the mother, particularly in domesticated animals who are consequently cared for by humans, may have a different effect on stress responses. For example, foals that were orphaned at birth and subsequently reared by humans were less stressed than mothered foals when tested in a novel environment when at least two months of age (Haupt and Hintz, 1983). Because orphaned kittens receive routine human handling during their basic care (e.g., feeding and stimulation to eliminate), we hypothesized that during the third week of life, like the foals, orphaned kittens would be less stressed, emitting fewer vocalizations and displaying less activity than mother-reared kittens.

Methods

All animal procedures were approved by the Animal Care and Use Committee at the University of California, Davis.

Subjects

Data were collected from May through August of 2019. We enrolled 50 kittens, and 49 kittens (28 orphaned, 21 mother-reared; 23 female, 26 male) from 11 litters (5 mothered, 6 orphaned) completed the study. Due to camera malfunction, no data were collected for one subject. Kittens were owned by three local rescue groups focused on neonatal kitten care and were cared for in the homes of human caretakers during the study. All orphaned kittens had at least one littermate and had been surrendered to rescue groups having been separated from their mothers. No kittens were deliberately orphaned for the purpose of this study. Mother-reared kittens were housed with and nursed by their mothers, and all but one had at least one littermate. Mothers were kept indoors (in a room) with their kittens and were not freely roaming. Orphaned kittens were bottle-fed a commercial kitten formula and cared for according to their respective rescue group protocols (kittens at this age should be fed every 2–4 hours). Both orphaned and mother-reared kittens were minimally handled by humans. At this age, kittens spend most of their time sleeping, so kittens are primarily handled for feeding, cleaning, weighing, or medical purposes.

Kittens were tested at approximately one and three weeks of age in their respective foster homes. For kittens who did not have a known date of birth, age was estimated by an experienced veterinary technician or rescue staff to the nearest approximated day upon intake based on a combination of teeth present, weight, degree of eye opening, and ear positioning (Little, 2011). Thirty of the kittens (10 orphaned, 20 mother-reared) were tested at both time points, with 2 additional orphans tested only at week 1, and 16 additional orphans tested only at week three. One mother-reared kitten was only tested at week 3. This resulted in a total of 79 trials (Table 1). For kittens tested at both time points, the testing sessions were conducted exactly two weeks apart. Attempts were made to keep time of day consistent between the two sessions to account for circadian rhythms and its potential effect on behavior.

Procedures

Each kitten was identified and given a designated number with minimal handling prior to testing. Some kittens were identified based on coat color, while others had colored safety collars on to assist in identification. One litter had identical coat colors with no collars, so a single uniquely-colored mark via non-toxic permanent marker (Sharpie®) was placed on the tip of their ear to aid in future identification. A random number generator ([google.com](https://www.google.com)) was used to randomize the order that kittens within a group would be tested for each session. Kittens' age, sex, and weight were recorded at each session. These were recorded after the session to minimize handling and separation of the kitten prior to the test. Immediately after recording these parameters, kittens were returned to their nest.

For each trial, each kitten was removed from their nest (mother and/or littermates) and placed individually in a 1-meter diameter pop-up nylon and mesh pen (Parkland Pet) in a separate room away from the rest of their litter for two minutes. Tests were conducted in foster homes, so there was variation in the distance between the testing arena and the nest. However, all trials were in a separate room as far away from the nest as possible, in a space free of other animals.

All kittens were unfamiliar with the researcher handling them for the testing sessions. All sessions were filmed for later coding, and two cameras (Kicktech Camcorder, Samsung Gear 360) mounted on tripods (Amazon Basics) were used to ensure all angles of the test arena were recorded at all times. Three pet training pads (Amazon Basics) were arranged to cover the floor of the test arena (see Figure 1). The pads were replaced between each individual kitten of a litter to remove any odor cues, and the test arena was fully disinfected with Rescue™ (Virox Animal Health, Ontario, Canada) between litters of kittens. Each kitten was handled with a clean pair of latex gloves (Curad).

The number of calls emitted and total activity (in seconds) were recorded for each kitten and every trial. A stopwatch (Adanac 3000, Marathon Watch, Ontario, Canada) was started as soon as the kitten was placed in the center of the test arena and the trial was ended after two minutes. See supplementary video (S1) for examples from trials with both orphaned and mother-reared kittens.

Video coding

All videos were coding using BORIS (Behavioral Observation Research Interactive Software; Friard and Gamba, 2016). Two behaviors were coded: each occurrence of a vocalization, and any time the kitten was active. The first three authors separately coded the same three videos to assess inter-rater reliability. Inter-rater reliability for vocalizations was substantial, average Cohen's kappa = 0.79 (range 0.61 – 0.91).

Because initial agreement between the three raters for activity was not consistently substantial or better (Cohen's kappa > 0.61), we further discussed kitten behaviors while watching multiple videos together. We then operationalized activity as any time the kitten spent engaged in these three behaviors: swaying/movement of the head only; displacement of the paws/body; and curling movement of the body, which entailed bringing the head closer to the posterior side of the body (separate from head swaying). Two of the authors (KL and SM) then coded six additional videos, at which point Cohen's kappa averaged > 0.61. The first author then coded all videos twice, and intra-rater reliability was substantial (average Cohen's kappa = 0.70, range 0.54 – 0.88).

Statistical Analyses

Analyses were conducted using R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) using the packages lme4 (Bates et al., 2014), nlme (Pinheiro et al., 2019), lattice (Sarkar et al., 2020), and ggplot2 (Wickham, 2016). Generalized linear mixed models (GLMM) were used to assess the effects of age, sex, and orphan status on activity and vocalizations, using data from all 79 trials. We also included interactions between orphan status with sex and age in both models. Kitten and litter ID were included as nominal random effects in all analyses, with kitten nested within litter ID, to control for repeated measures and any influence of being from the same litter. Both outcome variables were normally distributed (Shapiro-Wilk test, $p > .05$), although because vocalizations were integer-valued counts, we used a GLMM with a Poisson distribution for that analysis. Both models met the assumptions of normality (q-q plot) and homogeneity (Levene's test, $p > 0.05$) of the residuals. We used a Spearman's correlation test to assess the stability of

vocalizations and activity across the two tests in the 30 kittens (10 orphans, 10 mother-reared) for whom we had repeated data; and to assess whether there was a relationship between vocalization and activity.

Results

The means and confidence intervals for the means for active time and number of vocalizations for all comparisons are in Table 1. Orphaned and older kittens were more active, with an interaction between orphan status and age. Orphaned kittens showed a decrease in activity between weeks one and three, whereas mother-reared kittens showed an increase in activity between weeks one and three. There was no effect of sex, and no interaction between orphan status and sex.

Orphaned kittens emitted more vocalizations at both time points, and there was an interaction effect such that orphaned kittens emitted fewer vocalizations at three weeks of age compared to one week of age, but there was no change in mother-reared kittens. Table 2 details the results of the GLMMs. For kittens with repeated measurements, there was no correlation between vocalizations at weeks one and three ($r = -0.05$, $p = 0.79$), even when looking at mother-reared kittens ($r = -0.01$, $p = 0.96$) and orphaned kittens ($r = -0.11$, $p = 0.75$) separately. The activity measures from weeks 1 and 3 were correlated ($r = 0.50$, $p = 0.005$). There was no correlation between vocalization and activity ($r(79) = -0.12$, $p = 0.29$).

Discussion

In this study we found notable differences between the behaviors of orphaned and mother-reared kittens during a brief nest separation. Our first hypothesis was confirmed: orphaned kittens were more vocal and active during the open field test when they were approximately one week of age. Our second hypothesis was not supported, as we predicted fewer vocalizations and less activity among orphaned kittens compared to mother-reared kittens at three weeks of age. Orphaned kittens reduced their activity and vocalizations in week three compared to week one, whereas mother-reared kittens did not. However, orphaned kittens were still more vocal and active at both time periods in comparison to mother-reared kittens. The interaction effects we found support that the effects of orphan status on behaviors were moderated by age.

Because young animals often emit separation distress calls when isolated (Lingle et al., 2012), we interpret our findings to suggest that orphaned kittens experience more distress than mother-reared kittens when separated from their nest at both time points. The interpretation of increased activity by orphaned kittens is less clear. For our hypothesis, we expected kittens would be more active to increase their chances of relocating the nest, but locomotion is a rather complex behavior compared to vocalization. Some animals remain immobile when frightened, whereas others may increase activity (Hudson et al., 2017). Increased locomotion may also indicate an animal is more comfortable and willing to explore.

In a study of stress responses in infant rats, those who had been maternally-deprived but received human handling demonstrated greater exploration (Caldji et al., 2000). Although all

kittens in this study received human handling, orphaned kittens at this age are routinely handled briefly for feeding and to stimulate elimination six to 12 times per day, which is likely more human handling than most mother-reared kittens receive. This additional human handling could have increased orphans' exploratory behavior.

Locomotion requires complex coordination of multiple neuromuscular systems and develops more slowly in altricial animals (Hudson et al., 2017). Thus, an increase in activity as an animal ages would also be expected, as newborn kittens have much less motor coordination than three-week old kittens.

The differences in activity between orphaned and mother-reared kittens we observed in the first week is much more likely nest orientation than exploration, as kittens are both blind and deaf at that age, and highly reliant on tactile and olfactory cues to locate their mother (Larson and Stein, 1984). Thus, our results may indicate that orphaned kittens may be more motivated to relocate their nest than mother-reared kittens at one week of age. A previous study found that activity levels in mother-reared kittens increased in the second week of life when auditory and visual development is rapid, but then activity was stable until after the third week of life (Levine et al., 1980). At three weeks, kittens begin to play and show increased interest in littermates (Mendoza and Ramirez, 1987), suggesting alternative functions for locomotor activity at that age. Nest relocation would still be among the possible functions that could explain the increased activity in 3-week old orphaned kittens in the current study.

The effects of maternal deprivation on the later stress responses of animals have been frequently studied and well-documented (e.g., Francis and Meaney, 1999; Lueken and Lemery, 2004). These studies are frequently of rats or non-human primates and rarely include the effects of being orphaned and human-reared. Previous studies of animals who are human-reared or otherwise handled by humans early in life suggest that they may be less prone to stress when otherwise isolated. Under observation on pasture, there were no behavioral differences between mothered foals and those who were orphaned and hand-reared by humans. However, when isolated in a novel environment, mothered foals were more vocal and active than orphaned foals, which was interpreted as a sign of increased stress in the mothered foals (Haupt and Hintz, 1983). Rats who received daily human handling in addition to routine maternal care showed decreased fear and were more likely to eat and explore in a novel environment when compared to maternally-deprived and non-handled rats (Caldji et al., 2000).

In our study, orphaned kittens showed what appeared to be greater signs of stress during isolation than mother-reared kittens. The differences in results between horses and cats could be due to foals being precocial, whereas cats are altricial. Like cats, rats are altricial, but none of the rats in the aforementioned study were truly orphaned, and deprived rats only experienced maternal separation periods of 180 minutes/day (Caldji et al., 2000). The effects of being orphaned may be quite different from the effects of brief periods of maternal separation, as it is estimated that kittens spend at least 70% of their time in direct contact with their mothers for their first few weeks of life (Rheingold and Eckerman, 1971; Beaver, 2003). Although orphaned kittens are handled by humans for feeding and to stimulate

elimination, this handling likely cannot compensate for the lack of natural maternal care. However, maternal separation may not be the only variable leading to the group differences we found; orphaned kittens received more human handling, and may have also experienced less food competition, as mother-reared kittens develop nipple preferences and compete for access to their preferred nipple (Hudson et al., 2009). Bottle-feeding generally allows kittens to consume as much formula as they desire, without competition from siblings or disengagement by the mother.

Looking at individual consistencies in kittens for whom we had repeated measures, some results differed from a previous study where both vocalizations and activity in isolated kittens were consistent across testing sessions (Hudson et al., 2017). Like that study, we found a correlation between duration of activity in weeks 1 and 3 but we did not find a relationship between the number of vocalizations made in weeks 1 and 3. There are a few possible reasons for this disparity; because stable individual differences were apparent within the first 30 seconds of trials and were consistent across three-minute trials, we opted to shorten our testing trials to two minutes instead of three to reduce any potential distress to the kittens in our study. The previous study only tested mother-reared kittens, and we may have added variability to the data by including orphaned kittens. Because we utilized fostered animals for our subject pool, were uncontrolled factors that could have impacted the results, such as amount of human handling and actual time spent in contact with the mother.

Like Hudson et al. (2015) reported in both kittens and mice, we found no correlation between vocalizations and activity in kittens at either 1 or 3 weeks of age. This result provides further support for the possibility that the two behaviors have different underlying motivations or mechanisms; search or distress would not necessarily be mutually exclusive. It is also possible that either behavior indicates multiple motivations (e.g., both search and distress).

Our results suggest that the potentially negative effects of early maternal separation, in the form of increased distress vocalizations and possible search behavior or attempts to be reunited with the nest, continue even a few weeks after the initial separation from the mother. Future studies should determine if such differences in behavioral responses are observed in older orphaned and mother-reared kittens and cats, when kittens are typically independent of parental care. Other physiological and behavioral measures of stress and welfare should be incorporated to determine the effects of premature maternal separation in domestic cats (e.g., Delgado et al., 2020).

Conclusions

To sum, we found that orphaned kittens vocalized more and showed increased activity compared to mother-reared kittens during a brief nest separation at two timepoints: one and three weeks of age. Based on the understanding that these behaviors may indicate distress and search behavior, it is likely that being orphaned may increase stress responses in neonatal kittens. Further research should investigate the long-term behavioral responses of the domestic cat to premature maternal separation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We compared responses of orphaned and mother-reared kittens to a brief nest separation
- Orphaned kittens emitted more distress calls than mother-reared kittens
- Orphaned kittens were more active than mother-reared kittens
- Orphans were more vocal and active during a nest separation at 1 and 3 weeks of age
- Being orphaned likely increases stress responses of neonatal kittens



Fig. 1.
The experimental setup for trials.

Table 1.

Means, 95% confidence interval of the mean, and standard deviation for activity (s) and number of vocalizations by orphan status (OR=orphan, MR=mother-reared) and age group.

| Variable | OR 1 week (N=12) | MR 1 week (N=20) | OR 3 week (N=26) | MR 3 week (N=21) |
|---------------|----------------------|----------------------|----------------------|----------------------|
| Activity (s) | 69.26 [59.35, 79.17] | 30.62 [22.28, 38.96] | 59.05 [52.48, 65.62] | 39.70 [32.58, 46.81] |
| <i>SD</i> | 15.60 | 17.82 | 16.26 | 15.63 |
| Vocalizations | 70.08 [54.58, 85.59] | 63.15 [49.14, 77.16] | 66.73 [55.62, 77.84] | 63.81 [55.44, 72.18] |
| <i>SD</i> | 24.40 | 29.94 | 27.50 | 18.38 |

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Table 2.

Summary of Generalized Linear Mixed Models investigating the effects of orphan status, age and sex and interactions with orphan status on active time (s) and number of vocalizations emitted by kittens during a nest separation ($N = 79$ trials).

| Predictor | Estimate | CI of estimate | SE | <i>t</i> or <i>Z</i> | <i>p</i> |
|---------------|----------|-----------------|-------|----------------------|----------|
| Active time | | | | | |
| Intercept | 19.99 | [3.70, 36.28] | 8.03 | 2.49 | 0.018 |
| Orphan | 58.45 | [29.86, 86.04] | 12.64 | 4.63 | 0.001 |
| Age | 9.12 | [0.02, 18.23] | 4.45 | 2.05 | 0.049 |
| Sex | 2.79 | [-9.01, 14.59] | 5.82 | 0.48 | 0.635 |
| Orphan:Sex | 0.31 | [-16.27, 16.89] | 8.17 | 0.04 | 0.970 |
| Orphan:Age | -33.79 | [-19.64, -5.49] | 6.91 | -2.84 | 0.008 |
| Vocalizations | | | | | |
| Intercept | 4.09 | [3.81, 4.36] | 0.14 | 29.94 | <0.001 |
| Orphan | 0.47 | [0.08, 0.89] | 0.20 | 2.38 | 0.018 |
| Age | 0.02 | [-0.06, 0.10] | 0.04 | 0.45 | 0.654 |
| Sex | -0.04 | [-0.25, 0.18] | 0.11 | -0.33 | 0.740 |
| Orphan:Sex | -0.01 | [-0.31, 0.31] | 0.15 | -0.06 | 0.950 |
| Orphan: Age | -0.22 | [-0.35, -0.09] | 0.07 | -3.18 | 0.001 |