

Infant's Response to Social Separation Reflects Adult Differences in Affiliative Behavior: A Comparative Developmental Study in Prairie and Montane Voles

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As part of a comparative study of affiliative behavior, pups of two different vole species, *Microtus ochrogaster* (prairie voles) and *M. montanus* (montane voles), were compared for their responses to social isolation during the first 2 weeks of postnatal life. Previous studies have demonstrated that under both laboratory and field conditions, adult prairie voles show higher levels of affiliation than adult montane voles, although the species closely resemble each other morphologically. In the current study, prairie vole and montane vole pups showed profound differences in the behavioral and physiologic responses to social isolation. During 5 minutes of isolation, prairie vole pups emitted 314 ± 60 (days 4–6) and 601 ± 55 (days 8–10) ultrasonic vocalizations. At these same ages, plasma corticosterone increased 4–6 fold within 30 minutes of separation. The increase in corticosterone was highly correlated with the number of calls ($r = .66$). In contrast, most montane vole pups emitted no isolation calls under identical conditions. Moreover, montane vole pups had equivalent baseline corticosterone concentrations, but showed only a minor increase in corticosterone following separation at 4–6 days with no increase apparent at 8–10 days. As montane vole pups were capable of producing high levels of ultrasonic vocalizations and increased concentrations of plasma corticosterone in response to known stressors (tail suspension and halothane vapors), these results suggest that social isolation evokes less distress for montane vole pups than for prairie vole pups. The developmental difference in the amount of parent-young contact was not a sufficient explanation for the differences in the response to separation as cross-fostered montane voles failed to show an increase in ultrasonic vocalizations (although a slight increase in corticosterone was observed). Taken together, these studies indicate that species-typical

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adult patterns of affiliation may be apparent early in development in the response of infants to social separation.

Prairie voles (*Microtus ochrogaster*) and montane voles (*M. montanus*) are two closely related species of microtine rodents that display markedly different forms of social organization in the wild. Male and female prairie voles appear to form long-term monogamous relationships, share a nest and home-range, and may often live in extended family groups with more than one litter (e.g., Getz, Carter, & Gavish, 1981; Getz & Hofman, 1986). Montane voles, on the other hand, display a polygamous mating system; male and female montane voles occupy intrasexually exclusive territories and do not share a nest or home-range at any time of year (Jannett, 1980). A key proximate factor underlying this contrast in social dynamics appears to be a species-characteristic difference in the propensity for social contact. For example, when examined in the laboratory under identical conditions, adult prairie voles show much higher levels of affiliative behavior than do montane voles (Shapiro & Dewsbury, in press; Shapiro, Meyer, & Dewsbury, 1989). Moreover, pair bond formation following mating is characteristic of prairie voles but not montane voles (Carter, Williams, & Witt, 1990; Shapiro, Astin, Ward, & Dewsbury, 1986; Shapiro & Dewsbury, in press).

The neurobiology of affiliative behavior and social bonding has been the subject of increasing study in recent years (e.g., Reite & Field, 1985 and references therein; Carter, Williams, & Witt, 1990). However, despite much progress, there is still no general agreement on such fundamental issues as the definition and measurement of attachment, or on the relationship between the different forms of attachment bonds (Reite & Capitanio, 1985). One particularly important and still unanswered question concerns the ontogeny of social bonding. That is, the relationship between early social bonds, such as between mother and infant, and adult patterns of sociality (Reite & Capitanio, 1985). One of the most useful research strategies for exploring such questions involves the systematic comparative analysis of closely related species (Rosenblum & Kaufman, 1967). In the present work we examine the response to social separation in prairie and montane vole pups. Our goal is to trace developmental antecedents of those adult patterns of social bonding that differ so strikingly between these two species.

Infants of a wide variety of species display a characteristic "distress" or separation response following abrupt involuntary isolation from their parents and littermates (e.g., Allin & Banks, 1972; Hofer & Shair, 1978; Insel, Hill, & Mayor, 1986; Kaufman & Hinde, 1961; Panksepp, Herman, Vilberg, Bishop, & DeEskinnazi, 1980; Ritchey & Hennessy, 1987). Rodent pups normally respond to isolation by producing ultrasonic vocalizations in the 30–70 kHz range. These calls act as a potent stimulus to attract the mother and induce pup retrieval and/or maternal contact (Colvin, 1973; Hofer & Shair, 1978; Oswalt & Meier, 1975; Smotherman, Wiener, Mendoza, & Levine, 1977). In addition, maternal separation may be accompanied by activation of the hypothalamo-pituitary-adrenal (HPA) axis in guinea pigs (e.g., Hennessy & Ritchey, 1987) and in primates (e.g., Coe, Mendoza, Smotherman, & Levine, 1978).

In Experiment 1, we examine the effects of acute social isolation on ultrasonic vocalization and plasma corticosterone in 4–14 day old vole pups. Because adult prairie voles are significantly more contact prone than adult montane voles, we

tested the hypothesis that there was a parallel species difference in the strength of the response to social separation in development. In Experiment 2, we explore the role of experiential factors in generating species differences in the separation response by cross-fostering pups between species on the day of birth and testing their response to separation at 8 days of age.

Experiment 1

The purpose of this experiment was to determine if prairie vole and montane vole pups differ in ultrasonic vocalization or plasma corticosterone concentration following social isolation. Both of these measures change markedly across ontogeny, such that species differences in plasma corticosterone or ultrasonic vocalizations might simply be differences in the rate of development of the two species. To investigate this possibility, at the outset we observed litters from both species to describe the ontogeny of several developmental milestones. We then measured ultrasonic vocalizations and plasma corticosterone levels following social isolation in a separate group of animals. Finally, to control for the specificity of the separation response, two additional control groups were run. In the first, ultrasonic vocalization was recorded during a 60 sec period of tail suspension. In the second, plasma corticosterone concentrations were measured 30 min following brief exposure to halothane vapors.

Method

Subjects

Twenty-six prairie vole pups from 11 litters and 54 montane vole pups from 13 litters were scored daily for various postnatal developmental factors described below. In addition, 53 montane voles and 49 prairie voles were used to record ultrasonic vocalizations and plasma corticosterone concentrations. Animals were housed with both parents in clear polycarbonate cages (55 × 31 × 21 cm) with wood chips as bedding. Animals were maintained on a 14 L:10 D photoperiod with lights out at 1800 hr, and had continuous access to Purina rabbit chow and water. All animals were utilized in only one test throughout this study.

Procedure

Developmental Profile: Skin Temperature, Locomotion, Body Weight, Eye Opening, Fur Development, Incisor Appearance, Negative Geotaxis, Reflexive Surface Righting. Due to the extremely small size of the pups and the potential for injury, skin rather than rectal temperature was recorded. Pups were removed from the nest and temperature was immediately recorded from the surface of the ventrum (probe: YSI 420). Pups were then placed for 5 min in a polycarbonate open field with a 5 × 5 cm grid drawn on the floor. Ambient temperature of the apparatus was maintained at 24 ± 1°C. The number of grid cells entered by the pups during this time was also recorded. A grid crossing was counted when both front paws of the pup entered a new square. Following this 5-min period, skin temperature was again recorded to determine the temperature differential during

the 5-min period. Pup weights were monitored daily from postnatal day 1–21. In addition, we recorded the day on which both eyes were open, fur was visible, and incisors were erupted. To assess motor system development, pups were placed head downward on the midline of a 20 × 20 cm open field that was raised to a 35° angle. Animals were successful in completing the task if, in a given 60 sec trial, they turned a full 180° and faced up the incline. To assess the surface righting response, each pup was placed in a supine position and given 30 sec to return to a normal upright position.

Blood Sample Collection. Fifteen minutes before testing, home cages (parents and pups) were carefully transported from the colony room to the testing room. This procedure took approximately 30 sec and appeared to have little effect on the activity levels of the parents. The temperature of the testing room was maintained at 24° ± 1°C. Litter size for both species ranged from 2 to 5 pups with the majority being 3 or 4. In order to control for litter effects, one pup from each litter was used to obtain baseline values. Following the 15-min period of habituation in the test room, the control pup was immediately removed from the nest and rapidly killed by decapitation. Trunk blood was collected in 1.5 ml centrifuge tubes containing 5 µl of heparin. The samples were immediately centrifuged, plasma was then extracted and frozen until assayed for corticosterone. Iodinated corticosterone, a highly specific antiserum, standards, and assay protocol were provided by Radioassay Systems Laboratories, Inc., Carson, California. All samples were collected 3–7 hr after light onset.

Ultrasonic Vocalizations. Following the removal of the control pup as described above, vocalizations were then recorded for the remaining pups. To begin testing, a pup was removed from the nest and placed in a glass container (9 × 7 × 6 cm) with fresh bedding as substrate. The glass container was positioned within a polycarbonate recording chamber (46 × 29 cm). Vocalization was recorded during the first 5 min of separation with a microphone (Bruel and Kjaer Model 4385, Coopenhagen) suspended within a parabolic reflector approximately 10 cm above the pups. Vocalization signals were transformed by a digital sound spectrum analysis system providing on-line the number of calls in each 5-min session. Records were stored on disk to permit subsequent determination of peak frequencies (kHz) for each call (Burkholder, Hill, Vaughan, & Cascio, 1982). We determined the mean peak frequency of vocalization in each species by using the averages of 5 calls/pup for 4 prairie vole pups from 3 different litters and 5 montane vole pups from 3 litters. Following the 5-min recording period, each pup, still in the glass container, was carried to an adjacent room and remained there for an additional 25 min. Trunk blood was then collected and assayed for corticosterone as described above.

In addition, calls from a separate group of pups were subjected to further spectrographic analysis. Each pup was gently placed on a flat surface and calls were recorded using a Bruel and Kjaer microphone #4133 and a Nagra IV-SJ tape deck recording at 15 in/sec. Tapes were then played back into a Uniscan II real-time spectrograph. Representative sound spectrograms were printed using a Kay DSP sound spectrograph.

Tail Suspension. To determine if species differences in vocalization were the result of social isolation per se, or a nonspecific response due to an inability of one species to produce calls, pups ($n = 6$ prairie voles; $n = 9$ montane voles) were

suspended by the base of the tail approximately 10 cm from the microphone within the recording chamber and vocalizations were recorded for 60 sec. Peak frequencies were determined, as described above, for calls emitted during the tail suspension test. Five calls per pup were averaged from four montane voles and four prairie voles.

Halothane Stress. To determine if species differences in the corticosterone responses to social separation were simply the result of physiologic limitations of the HPA axis, pups of both species ($n = 5$ prairie voles; $n = 6$ montane voles) were exposed to a known stressor, halothane. Pups were removed from the nest and exposed to halothane vapors for 15 sec and then placed in $9 \times 7 \times 6$ cm glass containers with fresh bedding as substrate for 20 min. Blood samples were collected as described above and assayed for corticosterone.

Results

Postnatal Development

Table 1 lists various developmental parameters for each species. There were no species differences in the emergence of the geotaxic response or in the ability to demonstrate surface righting. However, there were significant species differences in the age at which both eyes were open, and when teeth and fur were visible. In all of these cases, prairie voles were the more precocious species. Weight gain for the two species is shown in Fig. 1. Weight gain for litters of both species is similar across the 21 day preweaning period. In order to determine if neonatal body weight was different between the species, days 1 and 2 were compared. Consistent with other developmental parameters reported here, prairie voles were significantly heavier on both days. For 1-day-old prairie voles, mean weight was 3.2 ± 0.09 gm ($n = 20$), vs 2.7 ± 0.03 gm ($n = 30$) for montane voles, $t(48) = 5.21$, $p < 0.0001$. Likewise on day 2, prairie voles weighed 3.5 ± 0.09 gm ($n = 20$) vs. 3.1 ± 0.05 gm ($n = 30$), $t(48) = 4.32$, $p < 0.0001$.

Skin temperature was no different for 2–3-day-old pups of either species when tested immediately following removal from the nest. There was also no difference in the change of skin temperature following a 5 min isolation in the polycarbonate open field. This was true even though fur had developed in prairie but not montane voles by day 3 (see Table 1). There was also no difference in the activity levels of the two species at 2–3 days of age. However, there was a significant difference in locomotion at 8–10 days of age. Prairie voles at this age were considerably more active than montane voles of the same age (Table 1).

As a crude assessment of developmental differences in the ability to thermoregulate, we recorded the age at which the change in skin temperature showed a 1°C or less change following 5 min isolation in the open field. As Table 1 shows, there was no difference in the age at which thermoregulation was evident.

Ultrasonic Vocalizations

Since many behavioral and physiological parameters change markedly during the first two weeks of age, species comparisons were made within three discrete age groups. Ultrasonic vocalizations for pups 4–6, 8–10, and 12–14 days of age

Table 1
Postnatal Growth and Development for Prairie and Montane Voles
 (Mean \pm SEM)

Measure	Prairie Voles	Montane Voles	t
Eyes open (age day)	8.5 \pm 0.6 (n = 17)	10.7 \pm 0.6 (n = 14)	9.57***
Incisors erupted (age day)	1.5 \pm 0.5 (n = 20)	3.5 \pm 0.5 (n = 10)	9.76***
Fur visible (age day)	1.7 \pm 0.2 (n = 9)	4.6 \pm 0.1 (n = 14)	13.98***
Negative geotaxis (age day)	6.5 \pm 0.3 (n = 12)	7.5 \pm 0.4 (n = 9)	1.79
Surface righting (age day)	5.2 \pm 0.6 (n = 13)	4.0 \pm 0.4 (n = 12)	1.39
Skin temp, °C (2-3 day olds)	31.2 \pm 0.46 (n = 7)	31.8 \pm 0.3 (n = 10)	1.15
Δ Temp following 5 min isol, °C (2-3 day olds)	1.9 \pm 0.3 (n = 7)	2.5 \pm 0.3 (n = 10)	1.23
Δ Temp following 5 min isol \leq 1°C (age day)	7.0 \pm 0.2 (n = 15)	6.9 \pm 0.4 (n = 10)	0.21
Locomotion, squares entered (2-4 day olds)	0.9 \pm 0.4 (n = 13)	0.0 \pm 0.0 (n = 10)	1.87
Locomotion, squares entered (8-10 day olds)	54.1 \pm 19.5 (n = 11)	5.8 \pm 1.2 (n = 17)	3.0**
Peak frequency isolation calls, kHz (6-8 day olds)	34.1 \pm 2.1 (n = 4)	34.9 \pm 1.1 (n = 3)	0.25

p based on unpaired t-test: **p < 0.005; ***p < 0.0001.

are shown in Fig. 2. Prairie vole pups 4–6 days of age exhibited a significantly higher rate of vocalization than did montane voles. Mean (\pm SEM) call rate for prairie voles was 314.9 ± 60.0 vs. 4.6 ± 3.7 calls/5 min for montane voles, $t(25) = 5.86$, $p < 0.0001$. The range for prairie voles was 2–625 calls; however, the majority of pups called at a rate of 200–300 calls/5 min. For montane vole pups, 7 emitted no calls at all, 6 were in the 3–5 call range, and 1 pup produced 30 calls in the 5-min test period.

This same species difference in call rate was evident at 8–10 days of age (Fig. 2). Mean call rate for prairie voles was 601.3 ± 55.6 ; for montane voles mean number of calls was 16.4 ± 13.8 calls/5 min, $t(20) = 12.9$, $p < 0.0001$. The range for prairie voles was 245–715 calls/5 min with the majority producing around 600 calls. Of the 14 montane voles, 9 produced no calls at all, 3 produced between 3 and 5 calls, and 1 pup produced 195 calls. It should be noted that prairie voles in both age groups were much more active than montane voles during the entire isolation period (see Table 1).

The time course of the vocalizations was not recorded. However, it appeared from monitoring the audible transduction of the individual calls that prairie vole

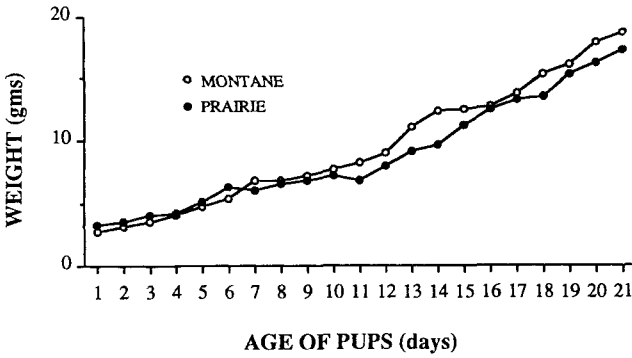


Fig. 1. Mean body weight during the first 3 weeks of postnatal life for prairie voles ($n = 18$ pups from 5 litters) and montane voles ($n = 28$ pups from 7 litters). Although rate of weight gain is similar between the two species, prairie vole pups weigh slightly more than montane vole pups at birth (see text).

calls began almost immediately and remained relatively constant over the 5-min test period. In addition, there appeared to be no litter effects in vocalizations. That is, there was more within- than between-litter variability in the pup call rate. Neither were there any overall order effects (sequence of removal from litter) in call rate.

Figure 3 shows a sonogram of a representative prairie and montane vole isolation call. The mean peak frequencies of the calls shown are slightly higher for the montane vole calls than for prairie voles (36 kHz vs. 33 kHz) although statistical analysis revealed no difference between peak frequency for 6–8-day-old prairie and montane voles (see Table 1). It can also be seen from Fig. 3 that intercall intervals are similar. However, there are interesting differences in the basic acoustic structure of the calls between species. The shape of the curves reveals a greater change in frequency within a shorter call duration for the montane pup (36–26

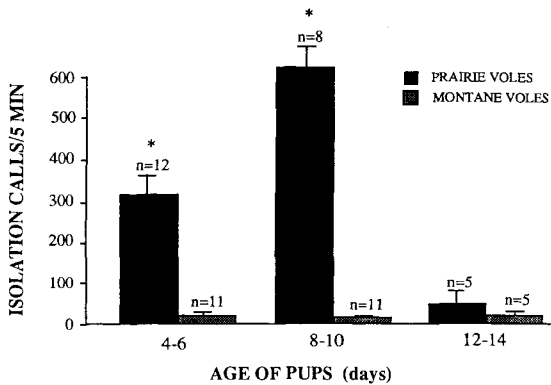


Fig. 2. Ultrasonic isolation calls for prairie and montane vole pups at three different ages. Data represent means (\pm SEM) for pups from at least three different litters at each age. Prairie vole pups call more than montane vole pups at days 4–6 and 8–10 ($*p < 0.001$ for species comparison). By days 12–14, after eyes have opened and locomotor behavior has increased, pups of both species gave few ultrasonic calls during social isolation.

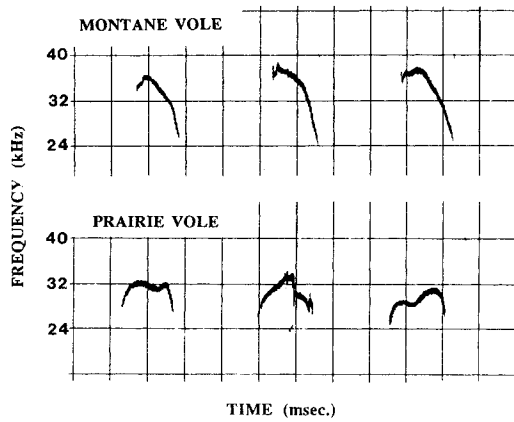


Fig. 3. Representative sonograms from a montane vole (9 days) and prairie vole (7 days) during social isolation. As most montane vole pups did not give isolation calls, several different pups were screened to produce this record. Note the difference in call morphology, with the montane call spanning a broader frequency range (36–26 kHz) although, in these records, both species show similar intercall intervals. Calls from both species during a brief interval of tail suspension resemble the patterns shown here.

kHz/0.125 sec for montane voles vs. 33–26 kHz/0.156 sec for the prairie vole pup). The rapid transient change in frequency and greater range of montane calls may be more localizable than the relatively flat ochrogaster calls (see Snowdon & Hodun, 1981). Although there is a certain degree of individual variability in call production, this pattern seems to be a fairly consistent species difference. Whether or not the call is invariant across different ages remains to be determined.

At 12–14 days of age, there was no significant difference in the vocalization rate between the species. Prairie voles called at a rate of 57.8 ± 33.0 calls/5 min, whereas montane voles produced 20.0 ± 12.3 calls/5 min. There was a large degree of within group variability at this age for both species. That is, 3 montane voles produced no calls while 2 produced 45 and 55 calls respectively. For prairie voles, 3 animals produced less than 10 calls whereas 2 produced 150 and 120 calls. At this age, there was also considerably more locomotion in both species (see Table 1). It should be noted that the eyes are open at this point and animals appeared to be exploring the apparatus.

Corticosterone Response

Figure 4 shows the plasma corticosterone concentration for the baseline and isolation conditions for the two species. Mean (\pm SEM) baseline values were 4.8 ± 0.5 ng/ml for 4–6-day-old montane voles and 5.0 ± 1.1 ng/ml for prairie voles. Following 30 min of isolation, prairie voles had a mean value of 31.2 ± 4.5 ; isolated montane voles had a value of 11.5 ± 1.4 . A two-way ANOVA (species \times condition) for 4–6-day-old pups revealed significant main effects for both species $p < 0.05$ and condition $p < 0.004$, with a significant interaction effect, $F(1, 27) = 5.51$, $p < 0.05$. Posthoc comparison of means (Sheffé F-test) revealed a significant difference only for the isolated prairie voles. However, within-species analysis

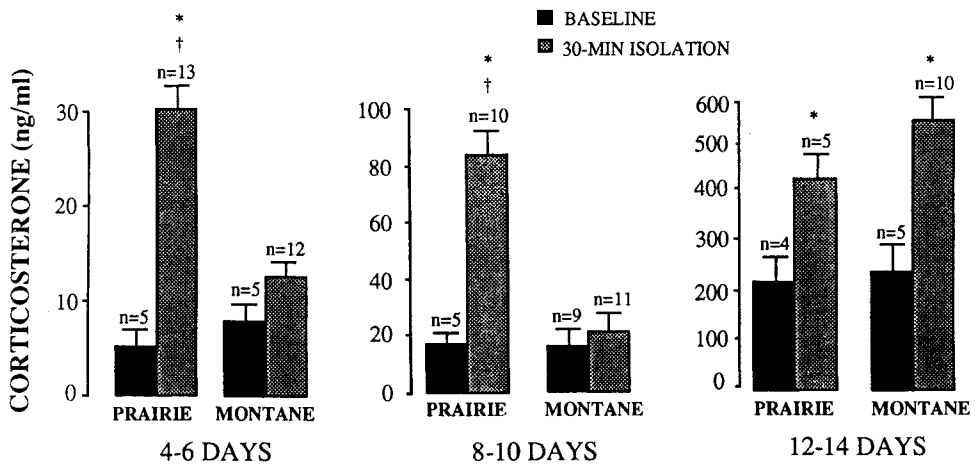


Fig. 4. Plasma corticosterone concentrations in prairie and montane vole pups at three different ages. Baseline corticosterone concentrations increase markedly across development, with both species showing equivalent patterns. At 4–6 days and 8–10 days, prairie vole pups show a four to six-fold increase in plasma corticosterone following 30 minutes of social separation (* $p < 0.05$ comparing separated to baseline conditions within species). This increase is greater than that seen in montane vole pups following an identical manipulation († $p < 0.05$ for species comparison).

revealed an effect for isolated vs. baseline montane pups $t(12) = 2.75$, $p < 0.02$. Thus, baseline levels for the species did not differ and both species showed increased corticosterone in response to separation, although the corticosterone response of the montane vole pups was less than that of prairie vole pups.

Mean baseline values for the 8–10-day-old pups was 14.2 ± 3.7 ng/ml for montane voles vs. 18.2 ± 9.7 ng/ml for prairie voles. Following 30 min of isolation, prairie voles had a value of 82.6 ± 11.7 vs. 20.6 ± 3.5 ng/ml for montane voles. Again, a two-way ANOVA (species \times condition) revealed significant main effects for both species and condition with a significant interaction effect, $F(1, 27) = 9.53$, $p < 0.005$. Posthoc comparison of means (Sheffé F-test) revealed a significant difference only for the isolated prairie voles. Unlike the case with 4–6-day-olds, however, within-species analysis revealed no effect for isolated vs. baseline montane pups $t(14) = 1.15$, NS. Thus, for 8–10-day-old pups, baseline levels did not differ and only prairie vole pups displayed a corticosterone response to separation.

Mean baseline levels for 12–14-day-olds was 237.3 ± 27.5 mg/ml for prairie and 580.4 ± 71.3 mg/ml for montane voles. Two-way ANOVA (species \times condition) revealed a significant difference for condition only with both species showing an increase in the isolated condition, $F(1, 20) = 8.33$, $p < 0.01$. There was no difference between isolated prairie and montane voles, $t(13) = -1.18$, NS. Thus, at this age, both species showed increased corticosterone concentrations in the isolated condition.

The relationship between the rate of vocalization and corticosterone levels for prairie vole pups ages 4–10 days of age ($n = 20$) are displayed in Fig. 5. There was a significant correlation between the number of vocalizations recorded in the first 5 min of separation and the plasma concentration of corticosterone 25 min later, $r = .66$, $p < 0.005$. A similar relationship prevailed when the age groups were

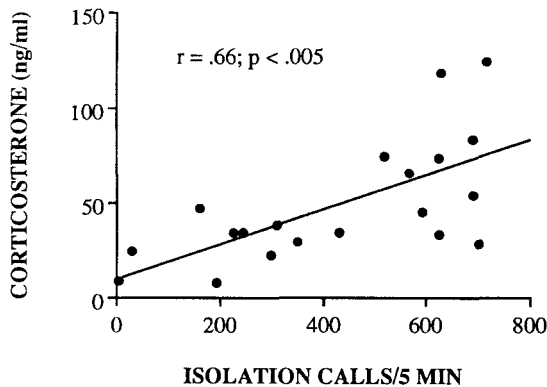


Fig. 5. Correlation of ultrasonic isolation calls recorded in the first 5 minutes of social separation and the concentration of plasma corticosterone measured at 30 minutes of separation in 4–10 day old prairie voles ($n = 20$).

analyzed independently, the 8–10-day-old animals displayed less of a correlation ($r = .40$, $n = 8$) than did 4–5-day-olds ($r = .61$, $n = 12$). As with call rate, there was no effect of order on corticosterone levels for any age group.

Control Groups

Tail Suspension. Figure 6 shows the vocalization response of 8-day-old pups to tail suspension. When suspended for 1 min, prairie voles vocalized at a rate of 162.5 ± 8.8 calls/min whereas montane voles produced 86.4 ± 5.2 calls/min. Comparison with the isolated condition reveals that prairie voles (8-day-old pups only) vocalized at a rate of 76.1 calls/min vs. 0.05 calls/min for montane voles. These results indicate that montane voles were able to produce ultrasonic vocalizations at this age and that the rate of calling was comparable to the rate for isolated prairie voles. In addition, two-way ANOVA (species \times condition) revealed no difference in the peak frequency of calls emitted during tail suspension vs. isolation either within or between species.

Halothane Stress. Following brief exposure to halothane vapors, prairie vole pups 8 days of age had corticosterone levels of 44.8 ± 4.7 ng/ml; montane vole pups had mean corticosterone levels of 64.6 ± 15.2 ng/ml (Fig. 6). Thus montane voles at this age are capable of showing a stress-related increase in plasma corticosterone that exceeds the isolation levels shown by prairie voles (54.8 ± 8.2 ng/ml).

Experiment 2

It has previously been shown that differences in the amount of tactile, thermal, and vestibular stimulation received by young animals are related to adult levels of sociality (e.g., Reite & Short, 1986, Scott, 1962; Wilson, 1982a,b). Happold (1967) reports a widespread correlation between the amount of parent-young contact experienced by young of various mammalian species and levels of contact proneness expressed in adulthood. What remains unanswered is to what extent this correlation is the result of experiential factors vs. genetic predisposition.

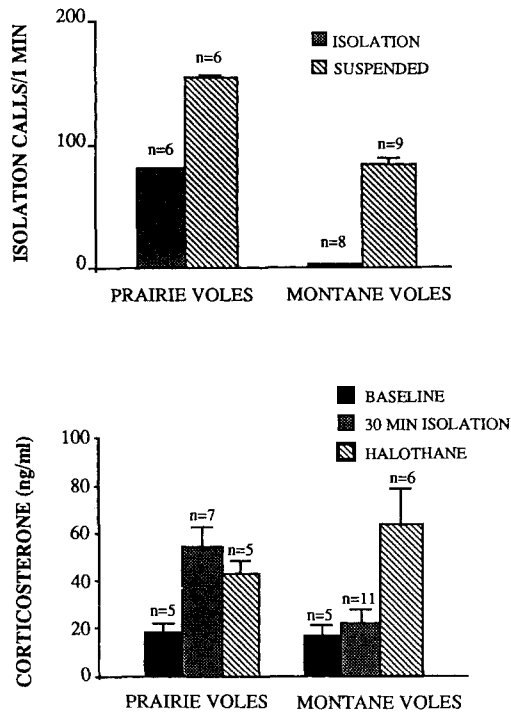


Fig. 6. Species differences are not as apparent in response to other stressors such as holding the pup by the tail for 1 minute (suspended) or halothane vapors. Both species (ages 4–10 days) show a significant increase in ultrasonic calls during tail suspension (data from 5 minute social isolation shown for comparison). Plasma corticosterone increases in both species (ages 8–10 days) in response to halothane vapors, although only the prairie voles at this age show a glucocorticoid response to social isolation.

In Experiment 2, we assessed the effects of parent-young contact on the generation of the marked species differences observed in Experiment 1. Previous reports have documented differences in the parental behavior of various species of *Microtus* (e.g., Dewsbury, 1985; McGuire & Novak, 1984, 1986; Oliveras & Novak, 1986; Wilson, 1982a,b). For example, in pup retrieval tests female prairie voles had significantly shorter latencies to retrieve, groom, nest build, and nurse pups than did female montane voles (Shapiro, 1987). In the present work, we first recorded differences in parent-young contact received by pups during the first 10 days of age. We then cross-fostered pups between species and recorded the response to social separation.

Method

Subjects

A total of 10 prairie vole breeding pairs and 12 montane vole breeding pairs were scored for parent-young contact. The number of pups involved in these observations was 54 prairie voles and 59 montane voles. Four litters of each species were utilized in the cross-foster manipulation. All breeding pairs used in this

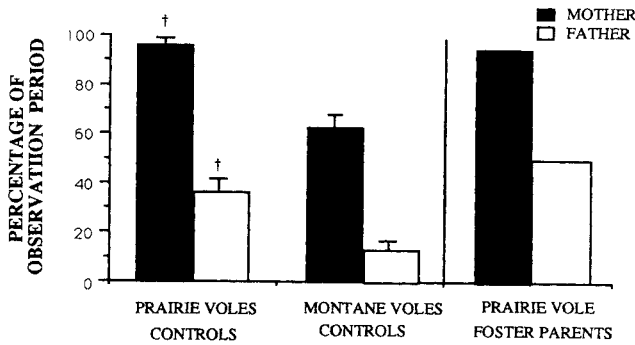


Fig. 7. Percentage of time parents are in contact with pups is shown for both species. Litters were observed in home cage from days 1–10, 10 min. twice a day. Prairie vole mothers and fathers spent more time in contact with their young than did montane vole parents ($\dagger p < .0001$). In addition, prairie vole parents spent equivalent time with fostered montane pups as with their own young. Attempts to cross foster prairie vole pups to montane parents were unsuccessful.

experiment had been in the colony for at least 2 months and all had given birth to at least 2 previous litters. Animals were maintained as described above.

Procedure

Parent-Young Contact. Parental behavior was scored twice daily in two 10-min observation periods. The first observation period was between 1000–1200 hr; the second was between 1500–1800 hr. Fifteen minutes before testing, home cages were removed from the rack in the colony room and placed on an adjacent observation table. Observations were made in the home cage so as not to disturb the animals. Time spent in contact with at least one pup was recorded for both the mother and the father. Contact was scored when the male or female were stationary and in direct contact with a pup for at least 5 sec. Duration was recorded by a single observer using a stopwatch.

Cross-fostering. Litters of pups were exchanged on postpartum day 0 or 1. Equal numbers of pups were transferred between parents with the smallest litter serving as the standard. The pups remained with their foster parents until they were 8 days of age. On the day of testing, home cages were transported to the testing room where the procedures for recording vocalization, isolating the pups for an additional 25 min, and collection of trunk blood were as described above. To ensure that there was no difference in the amount of parent-young contact received by fostered pups, two pairs of foster prairie vole parents were scored for parental behavior as described above. Controls for this experiment were nonfostered parents and offspring.

Results

Parent-Young Contact

Figure 7 shows the mean percentage of parent-young contact received by montane and prairie vole young for the total 10-day observation period. Prairie vole mothers spent significantly more time with their young than did montane vole

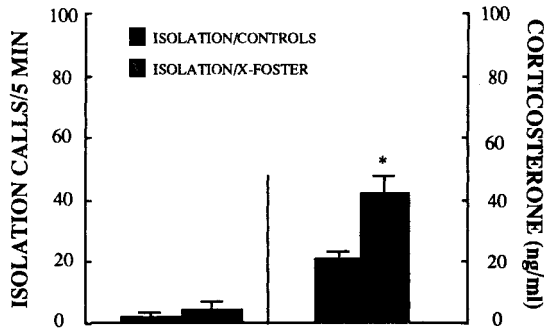


Fig. 8. Effects of cross-fostering on the montane vole's response to social isolation. At 8–10 days of age, montane pups still fail to emit ultrasonic vocalizations during 5 minutes of social isolation; however, increased concentrations of plasma corticosterone are evident following 30 minutes of separation.

mothers, 95.7 ± 1.4 vs. $62.2 \pm 5.2\%$, $t(18) = 6.13$, $p < 0.0001$ (unpaired t-test on the mean scores for days 1–10). Similarly, prairie vole fathers spent more time with their pups than did montane vole fathers, 35.5 ± 4.6 vs. $11.5 \pm 3.3\%$, $t(18) = 4.11$, $p < 0.0001$.

Cross-Fostering

A total of 4 litters from each species were cross-fostered. Consistent with past attempts to cross-foster these two species (Shapiro et al., 1989), prairie vole pups were not successfully reared by montane vole parents. Of the 4 litters that were crossed, only 1 survived until day 8. All of the montane litters crossed to prairie vole parents were successfully reared. To ensure that the amount of parent-young contact time experienced by the foster pups was similar to that received by control prairie vole litters, two litters of cross-fostered pups were observed from days 1–8 postpartum (see Fig. 7). The mean percentage of time spent by both prairie vole mothers was nearly identical to that of normals, 95.7% for normals vs. 96.6% for foster mothers; mean time spent with pups by foster fathers was 48.2% vs 35.5% for normals.

Figure 8 shows the response to isolation of these fostered pups. Fostered pups showed no difference from nonfostered pups in ultrasonic vocalizations, 0.45 ± 0.3 calls/5 min for normals vs. 6.5 ± 4.4 calls/5 min for fostered, $t(20) = -1.37$, NS. However, they did exhibit increased levels of corticosterone following separation. Mean response for normals was 20.6 ± 3.6 vs. 41.0 ± 6.2 for fostered pups, $t(20) = 2.85$, $p < 0.001$.

General Discussion

Separation Response

The present results have revealed a marked difference in the behavioral and adrenocortical response to social isolation by infants of two closely related species of rodents. Prairie vole pups placed in a test chamber began to emit ultrasonic

vocalizations within seconds and continued at high rates for the entire 5-min test period. The level of ultrasound production by prairie voles was similar to that reported for rat pups recorded in the same apparatus (e.g., Insel et al. 1986). Montane vole pups, isolated under identical conditions, produced very few calls. In fact, the majority of 4–10-day-old montane pups in this study produced no ultrasonic vocalizations at all during the 5-min test period. The absence of calls by isolated montane pups cannot be attributed to an inability to produce ultrasonic vocalizations. When suspended by their tails for 1 min, montane pups produced calls at a level comparable to prairie pups in the isolated condition. When they did produce calls, montane pups produced calls that are acoustically structured so that they may be easier to localize over a greater distance (Snowdon & Hodun, 1981) than are prairie vole calls. Localization might be less important for prairie pups since one or the other parent may always be within close proximity.

Activation of the HPA axis has long been thought of as a physiological indicator of stress of various kinds. It has also been associated with social separation in infant primates (Coe, Mendoza, Smotherman, & Levine, 1978) and guinea pigs (Hennessey & Ritchey, 1987). Consistent with results in other species, prairie vole pups 4–10 days of age displayed an increase in corticosterone levels over baseline when tested 30 min following social separation. Separated montane voles, on the other hand, displayed a relatively minor increase in corticosterone at 4–6 days of age and no increase at 8–10 days of age. Again, the lack of response in montane pups cannot be attributed to a species-characteristic physiologic immaturity of the HPA axis. Exposure to halothane vapors, a reliable stimulator of the HPA axis (e.g., Walker, Perrin, Vale, & River, 1986) produced an increase in corticosterone levels that was slightly higher than the increase recorded for isolated prairie voles of the same age.

There is much disagreement on the relationship between HPA activation and emotional distress (see Gunnar, Connors, Isenese, & Wall, 1988). Indeed, negative correlations have been reported between measures of behavioral distress and cortisol levels in humans (e.g., Rose, Jenkins, & Hurst, 1982; Gunnar, et al., 1988). In the present study, the rate of isolation calls and corticosterone levels were positively correlated, indicating that isolation calls might be a behavioral indicator, just as HPA activation may be a physiologic indicator, of distress in prairie vole pups. Taken together, the above results suggest that short-term social separation may be a potent stressor for prairie but not montane vole pups.

An interesting developmental trend is evident when one considers the response of 12–14-day-old animals to separation. At this age, neither species calls; however, both species show a corticosterone response. The eyes are open in both species by 12 days of age and locomotor activity is high. It thus appears that the corticosterone increase may represent a response to novelty rather than to social separation *per se*.

Cross-fostering

Hofer (1981) observed that, to the newborn infant, the mother *is* the environment. Viewed in light of differences in parent-young contact reported here, it is apparent that neonates of these two species experience considerably different early environments. Can the species difference in the separation response reported here for 4–10-day-olds be attributed to experiential factors? Although inconclu-

sive, the results of the cross-foster manipulation shed some light on this issue. Unfortunately, montane vole parents were unsuccessful in rearing prairie vole pups. The reason for this failure is unknown. Laboratory rats will readily foster other strains of rats or even mice (Deneberg, Paschke, Zarrow, & Rosenberg, 1969). In fact, montane voles have successfully fostered gray-tailed vole pups (McDonald & Forslund, 1978). However, these species were so closely related they are now considered one species (McDonald & Forslund, 1978).

There are several possible explanations for this phenomenon. Perhaps the fostered pups were rejected or even attacked by male or female montane voles. This does not appear to be the case. In fact, all of the litters were observed to be nursing by the end of postfoster day 3. A more likely explanation is that the failure of this cross was due to a species-typical difference related to nursing and/or milk delivery. When nursing, prairie vole pups attach to the nipple of the dam with such force that it is difficult to remove them by hand. In contrast, montane vole pups easily slip off of the nipple at the slightest touch. Therefore, following the transfer of pups, a montane vole dam can be seen moving about the cage dragging the attached prairie vole pups, a situation she no doubt rarely experienced with her own pups. Surprisingly, even though they had been on the nipple almost continually, prairie vole pups appeared to be underweight following 3 days of nursing with montane vole dams. It seems most likely, therefore, that the death of fostered prairie vole pups is related either to a mismatch between the prairie vole pup's pattern of suckling and the montane vole dam's milk delivery response (leading to inadequate nourishment) or to their ultimate rejection by the montane vole dam as a result of physical irritation, etc. In fact, very little is known regarding either the proximate mechanisms or adaptive significance of these curious species differences in milk delivery and nursing, phenomena that clearly warrant further study.

Montane vole pups, in contrast, were successfully reared by prairie vole parents. Fostered montane vole pups, as did nonfostered prairie voles, experienced nearly continuous thermotactile contact from their foster parents. Nevertheless, they produced virtually no ultrasonic vocalizations when isolated at 8 days of age. Interestingly, fostered montane pups did show an increase in plasma corticosterone concentrations following 30 min of isolation. Although this corticosterone response was not of the magnitude displayed by isolated prairie voles, it suggests an effect of continuous parent-young contact on HPA activation. This corticosterone response of fostered montane pups may represent a response to a novel environment, to social separation, or to changes in ambient temperatures. In addition, fostered pups may have been developing more rapidly such that the HPA axis became responsive slightly earlier. Since no in-foster controls were run, the response may also have been a result of the fostering process itself. Unfortunately, the present data do not allow us to discern between competing explanations. These results do, however, represent evidence that isolation calls and HPA activation can be dissociated in montane vole pups.

Postnatal Development

In the terminology of population ecology, a K-selected species is one with slow growth rates, small litter sizes, long life spans, and high parental investment in young. An r-selected species, on the other hand, is an opportunistic species that displays rapid population growth, large litter sizes, short life spans, and low

parental care (see Pianka, 1970). While classification along an r-K continuum is usually restricted to such broad categories as, say, rodents vs. primates, Nadeau (1985) and others have suggested that within the genus *Microtus*, prairie voles with smaller litter sizes and high levels of parental investment are K-selected relative to montane voles.

Curiously, the different profiles revealed in Table 1 are at odds with the above classification of these species. That is, one would expect a K-selected species such as the prairie vole to be the more altricial. Relative to montane voles, however, prairie voles are precocious with regard to such traits as eye opening, tooth eruption, fur, and body weight at birth. These results are also similar to those reported by Kruckenberg, Gier, & Dennis (1973) and Dewsbury (1981).

Taken together with the differences in separation response, these findings are consistent with the hypothesis of Pagel and Harvey (1990) that juvenile mortality is the salient force shaping such adaptations as precociality of young, increased parental care, etc. Given that joint parental care is adaptive for prairie voles, the rapid development shortly after birth, the strong response to separation, and the high levels of activity would insure that prairie pups would remain in close contact with their parents. In contrast, montane vole males and females do not cohabit and males do not contribute to parental care. Pups of this species are often left on their own for extended periods of time while the dam is out of the nest foraging. The production of ultrasound in this context would be maladaptive in that maternal retrieval is unlikely and the risk of alerting predators, many of whom can hear calls in the ultrasonic range (Colvin, 1973), would be greatly increased. Thus, if Pagel and Harvey (1990) are correct, one might expect a suite of traits such as a lack of isolation calls, low levels of locomotion, and relatively altricial development to covary within a species such as the montane vole.

Conclusions

Previous work has shown that the adrenocortical activity of preweanling rat pups can be inhibited by thermotactile contact with the dam (Stanton, Wallstrom, & Levine, 1987). This is convincing evidence for the potent regulatory effect on the pups' physiology of mother-infant contact (Hofer, 1978). Indeed, it appears that infant rats are born with a predisposition to find maternal contact reinforcing (Hofer, Shair, & Murowchick, 1989). This may also be the case for a wide variety of species, since it is the reinforcing value of contact comfort that is the critical force behind the generation of mother-infant attachment as well as other forms of social bonding (Stanton, Wallstrom, & Levine, 1987). What is remarkable in the present results is that they support the notion that contact comfort may not be inherently reinforcing for montane voles.

Rodent USV's have been variously described as cries of distress (e.g., Panksepp, et al., 1980), developmental predictors of adult fearfulness (Insel & Hill, 1987), signals for maternal retrieval (Oswalt & Meier, 1975), and acoustic byproducts of a thermoregulatory response (Blumberg & Alberts, 1989). Recently this behavior has been used to pharmacologically screen for anxiolytic drug potency (Gardner, 1985). Our results suggest that this behavior may reflect species-typical patterns of affiliation. Moreover, the present results highlight the fact that such

behavioral and physiologic phenomena as isolation calls and HPA activation following maternal separation are not ubiquitous in neonates. Rather they are adaptations that, as a result of different selection pressures, can vary considerably between even closely related species.

Notes

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