

Stimulus Control of Maternal Responsiveness to Norway Rat (*Rattus norvegicus*) Pup Ultrasonic Vocalizations

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Mother rats (*Rattus norvegicus*; 6 to 8 days postpartum) approach and maintain proximal orientation to a pup that is emitting ultrasonic vocalizations (USVs) far more than do virgin females (W. J. Farrell & J. R. Alberts, 2002). We used a playback regimen to examine the roles of acoustic and nonacoustic cues in regulating maternal proximal orientation toward vocalizing pups. When presented with recorded USVs, mothers of 6- to 8-day-old pups and nulliparous virgin females exhibited equivalent levels of proximal orientation toward the playback speaker. Mothers did show enhanced proximal orientation toward recorded USVs, however, if a silent pup was positioned below the speaker. Pup odors appear to be crucial for the maternal response to vocalizing pups, as peripherally induced anosmia attenuated maternal proximal orientation toward a vocalizing pup. Furthermore, spatial contiguity between olfactory and auditory stimuli was required for a maximal maternal response.

Parental behavior in mammals is an evolved system consisting, in part, of conserved features and species-specific specializations. Mother–infant interactions that constitute the basic nursing–suckling relations exemplify conserved features of mammalian maternal behavior. Likewise, the focus of the present article, mother rats' responses to infant rats' ultrasonic vocalizations (USVs), might be considered a specialization of Norway rats and other small rodents.

When removed from the nest and placed in a cool environment, infant Norway rats emit USVs. These vocalizations range from 30 to 50 kHz in frequency and hence fall outside of the human hearing range. Although these sounds are inaudible to humans, adult Norway rats can detect them (Brown, 1973; Crowley, Hepp-Reymond, Tabowitz, & Palin, 1965), and it is generally accepted that these sounds serve as a potent stimulus for maternal retrieval of pups (Allin & Banks, 1972), particularly when presented in conjunction with pup odors (Smotherman, Bell, Starzec, Elias, & Zachman, 1974; Smotherman, Bell, Hershberger, & Coover, 1978).

In our companion article (Farrell & Alberts, 2002), we introduced a behavioral assay to quantify maternal responsiveness to a vocalizing pup. Pregnant dams or mothers were observed simultaneously with matched virgin controls during exposure to a pup that was at first warm and silent. After this initial baseline period, the ambient temperature for the stimulus pup was lowered, and

equivalent observations of adults were made while the pup was cool and vocalizing. The major dependent variable was the amount of time adult rats spent oriented toward a mesh-covered hole adjacent to the stimulus pup. We refer to this behavior as *proximal orientation*, and the magnitude of the increase in proximal orientation while the pup was vocalizing served as an index of maternal responsiveness.

The results of our analysis of maternal development revealed that dams begin to show enhanced proximal orientation to vocalizing pups around the time of parturition. Maternal responsiveness continued to increase during the 1st week after birth and declined by the end of the 3rd week postpartum, approximately the time of weaning under standard laboratory rearing conditions (Thiels, Alberts, & Cramer, 1990). In subsequent induction experiments, a regimen of estrogen and progesterone administration designed to simulate certain aspects of the endocrine changes associated with the latter stages of pregnancy successfully induced enhanced responsiveness from virgin females relative to sham-operated controls. In contrast, long-term exposure to foster pups failed to elicit a similar increase in proximal orientation from virgin females, despite successfully inducing other forms of maternal responsiveness, namely retrieval and grouping of stimulus pups.

The results of our previous study establish a developmental trajectory for maternal responsiveness to a vocalizing pup that is similar to those previously reported for behaviors such as retrieval and grouping of pups (see Rosenblatt & Lehrman, 1963). In addition, the findings obtained from the induction experiments (cf. Experiment 2 in Farrell & Alberts, 2002) indicate that endocrine changes associated with pregnancy and parturition likely underlie at least the initial levels of maternal responsiveness to a vocalizing pup seen around the time of birth. It is important to realize, however, that responses seen in those experiments may not have been elicited exclusively or entirely by USVs per se. Rats in the previous study were exposed to a complex, multimodal stimulus: a vocalizing pup. In addition to hearing USVs, rats were exposed to nonacoustic stimuli, including pup odors.

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Maternal responses to recorded USVs have been studied with a variety of methodologies, and there have been varying results regarding the extent to which USVs elicit maternal approach in the absence of additional nonacoustic pup cues. In the present study, we sought to determine the extent to which nonacoustic pup cues play a role in our paradigm of eliciting an enhanced maternal response to vocalizing pups.

We first examined the responses of mothers of 6- to 8-day-old pups and of virgin controls to presentation of recorded USVs either in the absence or presence of additional nonacoustic cues provided by a warm, silent pup. We next examined the role played by olfactory stimuli by comparing the responses of anosmic and control mothers during exposure to stimuli from a live vocalizing pup. Finally, we dissociated the source of USVs (recorded) from the source of pup odors (provided by a silent pup) to determine whether pup odors provide mothers with directional information used when responding to USVs.

Experiment 1: Maternal Responses to Recorded USVs

Research conducted to date has yielded mixed results regarding the extent to which recorded USVs elicit maternal approach in the absence of additional nonvocal pup cues. Allin and Banks (1972) tested mothers of 6- to 9-day-old pups, virgin females, and males for responses to recorded USVs, using a large, hexagonal arena containing a central nestbox covered by a circular roof. Although the dimensions of the arena were somewhat vaguely given, the distance between the nestbox and the sides of the arena appears to have been ≈ 60 – 70 cm. During test sessions, sounds were presented randomly from each of the six walls of the arena. Each rat heard either recorded USVs or tape noise. Head movements during sound presentation suggested that mothers localized recorded USVs more accurately than did virgins. Surprisingly, most males also oriented toward the sounds at better than chance levels. The addition of pups to the nestbox did not appear to alter systematically the frequency or accuracy of the head orientations displayed by mothers, indicating that nonvocal pup cues were not necessary for this response to USVs. Unlike virgins and males, mothers frequently responded with a sortie to recorded USVs. That is, they exited the nestbox and approached one of the walls of the apparatus. The majority of maternal sorties were directed toward the source of the recorded USVs, indicating localization. Unlike recorded USVs, recordings of background noise tended to elicit random orienting responses and rarely elicited sorties.

Allin and Banks's (1972) results suggest that mothers orient toward and approach recorded USVs regardless of additional, nonacoustic cues from pups. Smotherman et al. (1974) reported, however, that pup odors are required for accurate maternal approach toward USVs on a Y maze (mothers were tested daily on Postpartum Days 9–13). When one arm of the maze was left empty and recorded USVs were played from a speaker located at the end of the opposing arm, mothers entered the two arms at chance levels. When a comatose pup was positioned at the end of the previously empty arm, however, mothers reliably entered the arm containing the ultrasound speaker. In part on the basis of these findings, Smotherman et al. (1974) concluded that pup odors are required for mothers to approach USVs. Presumably, pup odors act as a general potentiating stimulus for maternal retrieval, and USVs

provide mothers with directional information that guides their response.

The methodology for assessing female rats' responses to vocalizing pups introduced in our companion article (Farrell & Alberts, 2002) offers a number of features that make it advantageous for examining responses to recorded USVs. First, our small observation cages facilitate rapid exploration and habituation, precluding the necessity for extensive habituation (sessions spread over multiple days) to the apparatus. The small size of the observation cages also distinguishes our procedure from open-field tests, which are typically used to measure psychological constructs such as fearfulness and timidity. Finally, our apparatus and procedures can be used to examine responses to either vocalizing pups or recorded USVs without altering key aspects of the test situation.

Given the numerous differences between our behavioral assay and the playback paradigms used by other researchers, it was necessary to examine in our setting the respective roles played by acoustic and nonacoustic pup cues. To do this, we conducted a series of playback experiments during which mothers and virgin females were exposed to recorded USVs either with or without a warm, silent pup positioned beneath the playback speaker. Given the inconsistencies in previous reports regarding responses to recorded USVs presented alone, it was unclear whether nonacoustic pup stimuli would be required for mothers to display an enhanced response to recorded USVs relative to virgin controls. It was expected, however, that the presentation of nonacoustic pup stimuli in conjunction with recorded USVs would enhance mothers' responses to the recorded vocalizations.

Method

Subjects. A total of 84 adult female Sprague-Dawley rats (*Rattus norvegicus*) and 20 stimulus pups (6 to 8 days of age) were used in the current study. There were 38 adult virgin females, 20 mothers of 0- to 1-day-old pups, and 26 mothers of 6- to 8-day-old pups. Animals were housed in standard polycarbonate cages ($45 \times 25 \times 20$ cm) lined with shredded aspen bedding and were maintained on a 12-hr light–dark cycle. Litters were culled to 8 pups on Postpartum Day 3 (day of birth = Day 0). Ambient temperature was maintained at 22 ± 2 °C, and food and water were available ad libitum.

Apparatus. Testing for maternal responsiveness to recorded USVs was conducted in a modified version of the apparatus described elsewhere (Farrell & Alberts, 2002). Briefly, the apparatus included a single, clear Plexiglas observation cage ($32 \times 22 \times 23$ cm) positioned 10 cm from an ultrasound speaker (Ultrasound Advice, London, England) attached to a ring stand. A mesh-covered hole (10.2 cm diameter) in the side of the observation cage faced the playback speaker, and a second mesh-covered ventilation hole (7.6 cm diameter) was located on the opposing wall. Both the observation cage and the ultrasound speaker were elevated 10 cm above the countertop. When required, a warm silent stimulus pup could be positioned directly beneath the playback speaker. The ambient temperature surrounding the stimulus pup was maintained at ≈ 35 °C with the aid of an open-ended chamber connected to a thermostatically controlled water bath (see Farrell & Alberts, 2002).

The only illumination in the testing room was provided by a reflective lamp outfitted with a 7.5-W red lightbulb. This lamp was positioned 9 cm above the center of the observation cage. Output from the lamp was attenuated by 30% using a variable voltage transformer, thereby providing minimal light levels required for video recording. A black-and-white video camera with infrared sensitivity (Panasonic, BL200) and a time-lapse videocassette recorder (VCR; Gyr, TLC 1400) were used to record experimental sessions.

USV stimuli were constructed from 56 sound clips recorded from 39 pups (6 to 8 days of age). Stimulus vocalizations were elicited by cold exposure (15 °C). Recording was conducted in a sound-attenuating chamber (Lehigh Valley Electronics, Philadelphia, PA; 56 × 37 × 40 cm) using a microphone and associated preamplifier, with a flat frequency response (± 2 dB) from 4 Hz to 100 kHz (Bruel & Kjaer, Model # 4135). The microphone was positioned 10 cm from the head of the stimulus pup at 0° incidence. Output from the microphone and preamplifier was passed through a precision amplifier (Bruel & Kjaer, NEXUS conditioning amplifier) to a portable ultrasound processor (Ultrasound Advice, London, England) containing a high-speed analog-to-digital converter. Sounds were digitally recorded in the portable ultrasound processor (PUSP) using a sampling rate of 228 ks/s. Sound clips were then replayed at one-tenth normal speed and were subsequently passed to a Power Macintosh 7600 computer, where they were recorded, analyzed, and archived using Canary software (Cornell Bioacoustics Workstation, Ithaca, New York). Noise with a frequency above 100.0 kHz or below 1.7 kHz was filtered out of stimulus recordings using the Canary software.

Stimulus recordings contained a minimum of 2 and a maximum of 23 USVs (minimum gap of 25 ms between sounds). The amplitudes of the recorded USVs were variable: low-intensity USVs could barely be detectable above the noise floor (≈ 49.8 dB), whereas high-intensity vocalizations had peak sound pressure levels as high as 88 dB. The frequencies of the recorded vocalizations typically fell between 34 and 60 kHz.

Playback was accomplished by replaying the slowed down sounds from the computer and passing this output to the PUSP. The recordings were then time compressed, yielding a signal with the timing and frequency characteristics identical to the original recorded USVs. Output from the PUSP was passed through an ultrasound amplifier (Ultrasound Advice, London, England) to the ultrasonic speaker located adjacent to the observation cage. Output from the amplifier was also passed to an oscilloscope that provided visual confirmation of playback. Speaker output was verified with the aid of a commercially available ultrasound detector (Ultrasound Advice, Model 430, London, England).

Procedure. Subjects (either a mother rat or virgin female rat) were allowed to habituate to the observation cage for 40 min. Behavior was then video recorded during a 20-min baseline period (silent) and a subsequent 20-min test period (USV playback). During the test period, rats were presented with four different sound loops constructed from the repeated presentation of a single stimulus recording. Each sound loop was presented four times in succession, with a 30-s interstimulus interval (silence). The fourth presentation of each sound loop was followed by 1.5 min of silence. During these longer pauses, a new stimulus recording was downloaded to the PUSP for playback. The final sound loop was allowed to run for 1.5 min after the fourth presentation, bringing the total duration of the test period to 20 min.

Testing was conducted using three different stimulus configurations. In two separate experimental comparisons, the behavioral responses of mothers of 0- to 1-day-old pups ($n = 12$) and mothers of 6- to 8-day-old pups ($n = 8$) were compared with those of matched virgin controls ($n = 12, 8$) during exposure to recorded USVs replayed at original intensity. In both of these experimental comparisons, mothers and matched virgin controls were exposed to the same acoustic stimuli. The behavioral responses of mother and virgin female rats were also examined during exposure to recorded USVs that were attenuated by 20 dB. In this comparison, a single set of sounds was presented to 8 mothers of 0- to 1-day-old pups, 8 mothers of 6- to 8-day-old pups, and 8 virgin female rats.

Finally, the behavioral responses of mothers of 6- to 8-day-old pups ($n = 10$) were compared with those of a set of matched virgin controls ($n = 10$) during exposure to recorded USVs presented in conjunction with non-acoustic pup stimuli. In this portion of the experiment, a warm silent pup was positioned under the playback speaker during the habituation, baseline, and test periods. The same recorded acoustic stimuli were presented to both

mothers and matched virgin controls. All USVs emanated from the speaker. The stimulus pup remained silent in all phases of the experiment.

Video recordings were coded for the duration of proximal orientation displayed during both the baseline and test periods. Proximal orientation was registered whenever rats were actively oriented toward the playback speaker, with their nose in close proximity (< 5 cm) to the mesh-covered hole adjacent to the speaker. Proximal orientation was only registered during active orientation: Merely sleeping while in close proximity to the playback speaker did not qualify. All behavioral coding was performed with the aid of customized DOS-based software.

Data analysis. The duration of proximal orientation displayed by each mother or virgin control rat during the baseline period was subtracted from the duration during the subsequent test period, yielding difference scores that reflected the change in proximal orientation elicited by USVs. Data are presented as mean (\pm SEM) values of these difference scores for each group of mothers and virgin females. Differences between the mean difference scores obtained from mothers and virgin controls were tested for statistical significance using matched pairs *t* tests, as individual pairs of mothers and virgins were exposed to the same sets of stimulus recordings. Paired *t* tests were also used to determine whether mothers and virgin control rats displayed significant increases in proximal orientation during exposure to full-intensity USV recordings (comparisons against baseline). Differences between means were considered to be statistically significant if $p < .05$ (two-tailed).

Results and Discussion

Playback at original intensity. Recorded USVs presented at original intensity failed to evoke an enhanced maternal response, as can be seen in Figure 1. During the baseline period, mothers of 6- to 8-day-old pups and matched virgin controls engaged in an average of 12.8 ± 8.5 and 54.0 ± 25.3 s of proximal orientation,

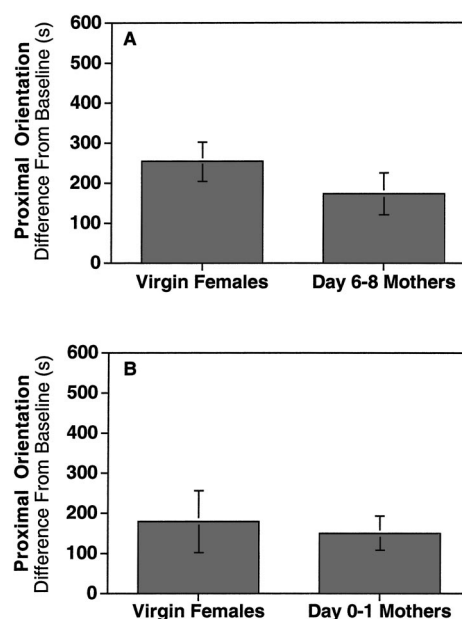


Figure 1. Duration of proximal orientation by mothers of 6- to 8-day-old pups (A), mothers of 0- to 1-day-old pups (B), and matched virgin controls in response to recorded (original intensity) ultrasonic vocalizations (USVs). Histograms represent the mean difference between the 20-min test period (USV playback) and the 20-min baseline period (silence). Error bars represent the SEM.

respectively. During USV presentation, mothers of 6- to 8-day-old pups exhibited an average increase in proximal orientation of 174.0 ± 51.3 s. The average increase in proximal orientation displayed by virgin rats during the test period was 254.3 ± 48.0 s. The mean increases in proximal orientation displayed by mothers of 6- to 8-day-old pups and matched virgin controls did not differ significantly, $t(7) = 1.13$, $p < .29$.

The equivalence of mothers' and virgins' responses to recorded USVs cannot be explained by changes in mothers during the 1st week postpartum. Figure 1B shows that mothers of newborns and matched virgin controls also displayed equivalent increases in proximal orientation during USV exposure. During the baseline period, mothers of newborns and matched virgin controls displayed an average of 32.5 ± 12.7 and 163 ± 44.4 s of proximal orientation, respectively. On average, mothers of newborns engaged in an additional 149.5 ± 43.3 s of proximal orientation during USV playback. Virgins, however, also displayed an increase in proximal orientation during the test period, yielding a difference score of 179.5 ± 76.2 s. The results of a paired t test indicated that the mean increases in proximal orientation displayed during USV exposure did not differ significantly between groups, $t(11) = 0.38$, $p < .71$.

Attenuated (-20 dB) playback. Attenuated recordings of USVs also failed to evoke different responses from mothers and virgins, as can be seen in Figure 2. During the baseline period, virgin controls displayed an average of 107.3 ± 30.5 s of proximal orientation. On average, mothers of newborns and mothers of 6- to 8-day-old pups spent 22.5 ± 8.8 and 45.8 ± 22.1 s of the baseline period engaged in proximal orientation, respectively. All three groups displayed similar average increases in proximal orientation during playback of attenuated USVs: Virgins engaged in an additional 120.8 ± 40.9 s of additional proximal orientation, whereas mothers of newborns and mothers of 6- to 8-day-old pups displayed increases of 118.5 ± 20.1 and 103.5 ± 58.8 s, respectively (Figure 2). There were no statistically significant differences between the mean increase in proximal orientation displayed by

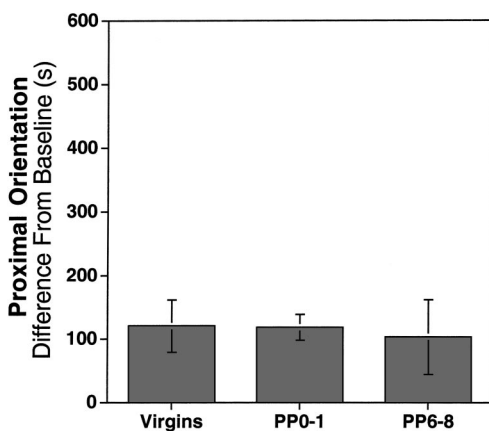


Figure 2. Duration of proximal orientation by virgins and mothers on Postpartum Days 0-1 (PP0-1) and 6-8 (PP6-8) in response to attenuated (-20 dB) recordings of ultrasonic vocalizations (USVs). Histograms represent the mean difference between the 20-min test period (attenuated USV playback) and the 20-min baseline period (silence). Error bars represent the SEM.

virgin females and those displayed by either group of mothers, $t(7) = 0.05$, $p < .97$ and $t(7) = 0.27$, $p < .79$, for mothers of newborns and mothers of 6- to 8-day-old pups, respectively.

Playback with a warm silent pup. Although mother and virgin rats responded similarly when presented with recorded USVs alone, recorded USVs presented in conjunction with the nonacoustic cues provided by a warm silent pup did elicit enhanced maternal responses. Figure 3 shows the dramatic difference between dams and virgins. On average, mothers of 6- to 8-day-old pups and matched virgin controls spent 69.0 ± 16.1 and 91.2 ± 36.2 s of the baseline period engaged in proximal orientation, respectively. When recorded USVs were replayed from the ultrasound speaker in the presence of the nonacoustic stimuli provided by the silent pup, mothers of 6- to 8-day-old pups responded with a mean increase in proximal orientation of 376.2 ± 69.0 s. In contrast, virgins exhibited an increase of only 118.8 ± 53.5 s in proximal orientation (see Figure 3). A paired t test comparing the mean increases in proximal orientation displayed by mothers and virgins revealed a statistically significant difference between the two groups, $t(9) = 2.94$, $p < .05$.

The present results indicate that nonacoustic stimuli associated with an infant rat modulate the mother rat's response to the pup's USVs. Mothers displayed enhanced increases in proximal orientation only when stimulus recordings were presented in the presence of a silent pup. In contrast, virgin females' responses were unpotentiated to the combination of USVs and nonacoustic cues. The current findings are consistent with previous reports indicating that pup odors enhance maternal approach to USVs on a Y maze (Smotherman et al., 1974, 1978). The configuration of the testing apparatus used in the present study, and the limited lighting in the testing room, led us to suspect that odors are likely to be the crucial nonacoustic stimuli perceived by mothers. This hypothesis was tested in Experiment 2 using an anosmia procedure.

Though nonacoustic stimuli emanating from a pup clearly enhance maternal responsiveness to USVs, the present findings do not support the conclusion of Smotherman et al. (1974) that pup odors are necessary for mothers to reliably approach USVs. Examination of the magnitude of the increases in proximal orientation during USV playback (full intensity) reveals that both mothers and virgins approach and orient toward the recorded USVs. When data for both of the playback studies (full-intensity sounds) are collapsed, paired t tests indicate that both mothers and virgins displayed significant increases in proximal orientation during USV exposure, $t(19) = 4.9$, $p < .01$, and, $t(19) = 4.3$, $p < .01$, for mothers and virgins, respectively. Adding nonacoustic pup cues to USV playback enhanced mothers' responses to recorded USVs; the same additions may also have dampened modestly the virgins' responses, yielding behavioral differences between mothers and virgins.

The finding that mothers and virgins displayed equivalent increases in proximal orientation when recorded USVs were presented alone is also inconsistent with the report of Allin and Banks (1972), which suggested that mothers orient toward recorded USVs more accurately than do virgins and that mothers are more likely than virgins to respond to recorded USVs by leaving the nest and approaching the source of recorded USVs. Methodological differences may underlie this discrepancy. Specifically, the size and configuration of the apparatus used by Allin and Banks (1972) might have inhibited virgins from leaving the nest and approaching

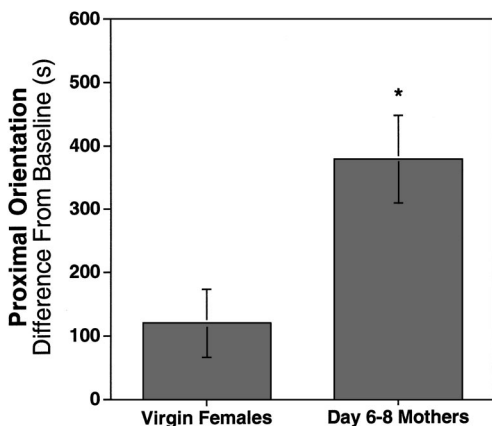


Figure 3. Duration of proximal orientation by mothers of 6- to 8-day-old pups, and matched virgin controls during exposure to recorded ultrasonic vocalizations (USVs) and nonvocal pup cues. Histograms represent the mean difference between the 20-min test period (USV playback + silent pup) and the 20-min baseline period (silent pup). Error bars represent the SEM. Statistical comparisons were made against matched virgin controls (* $p < .05$).

the source of the recorded USVs in their paradigm. The testing arena used by Allin and Banks (1972) was much larger and more brightly illuminated than the one used in our experiments. Fleming (1986) has suggested that the hormonal changes associated with pregnancy make mother rats less timid than virgins. This suggestion has been subsequently supported by the finding that ovariectomized virgin females treated with estradiol and progesterone enter an open field more rapidly than do control virgins (Fleming, Cheung, Myhal, & Kessler, 1989). These virgins also displayed elevated levels of ambulation in the test arena after steroid administration (Fleming et al., 1989). Citing an inability to get virgins to settle in the covered nestbox in the center of their large hexagonal arena, Allin and Banks (1972) noted that additional lights were turned on in the room during tests involving virgin females. The additional illumination may have made the apparatus even more fear evoking for virgin females and hence inhibited them from leaving the nest during USV playback.

Experiment 2: Maternal Responsiveness to a Vocalizing Pup After Peripheral Induction of Anosmia

The results of the previous experiment demonstrate that non-acoustic pup cues co-act with USVs to elicit an enhanced maternal response. It seemed highly unlikely that visual cues from the stimulus pup were involved because of the physical arrangement of the apparatus and because testing was conducted in a darkened room with only modest levels of red light. A more compelling explanation was that odors emitted by the silent pup interacted with recorded USVs to elicit an enhanced maternal response. Nevertheless, because nonacoustic cues other than pup odors were not systematically eliminated when recorded USVs were presented in the presence of the warm silent pup, the possibility still remained that mothers might also have been attending to nonolfactory cues as well as to pup odors.

The present experiment was designed to examine more closely the relationship between olfaction and maternal responsiveness to USVs. Specifically, we wanted to find out whether anosmia would alter mother rats' responses to a live, vocalizing pup. To examine this question, we simultaneously observed the responses of anosmic and intact mothers of 6- to 8-day-old pups during exposure to a stimulus pup that was either warm and silent or cool and vocalizing. We used a single vocalizing pup as the stimulus source because previous research has established that sensory cues associated with littermates attenuate USV production (Hofer & Shair, 1980, 1987, 1991).

To avoid nonolfactory confounds associated with procedures such as olfactory bulbectomy (see Alberts & Friedman, 1972), anosmia was induced by perfusing the nasal cavity with $ZnSO_4$ solution. This procedure is known to produce a temporary state of anosmia by acting at the level of the sensory epithelium (Alberts & Galef, 1971). Given the results of the previous experiment and Smotherman et al.'s (1974, 1978) findings indicating that pup odors enhance maternal approach toward recorded USVs in the Y maze, it was anticipated that anosmia would attenuate mothers' responses to a vocalizing pup.

Method

Subjects. The present study used 16 Sprague-Dawley rat (*Rattus norvegicus*) mothers of 6- to 8-day-old pups. Eight stimulus pups (6 to 8 days old) were used to provide both acoustic and nonacoustic stimuli. Rats were housed under the conditions described in Experiment 1.

Apparatus. For a complete description of the apparatus used to test responsiveness to a vocalizing pup, please refer to the companion article (Farrell & Alberts, 2002). Briefly, this apparatus consisted of two Plexiglas observation cages similar to that used in Experiment 1 and an open-ended environmental chamber used to house stimulus pups. Observation cages were positioned 10 cm from both of the opened ends of the environmental chamber. Ambient temperature inside the environmental chamber, which was constructed from a double-walled glass cylinder, could be controlled precisely by passing water from a thermostatically controlled water circulator between the inner and outer walls of the chamber. Each observation cage was illuminated with a reflective lamp and a 40-W light located 9 cm above the perforated steel roof of the cage. A 52-W bulb located 1.93 m above the observation cages provided additional light. A piece of cardboard was placed on the roof of each observation cage to shade the half of the cage located farthest from the stimulus pup. USVs emitted by the stimulus pup were monitored with the aid of a commercially available ultrasound detector, the microphone for which was placed at a 45° angle to one of the opened ends of the environmental chamber. The bat detector was set to detect sounds in a frequency range centered on 43 kHz.

Procedure. Behavioral responsiveness to a vocalizing pup was assessed using the procedures of Farrell and Alberts (2002). Eight mothers of 6- to 8-day-old pups were tested 24 hr after having been rendered anosmic. Saline-perfused mothers ($n = 8$) served as matched controls.

At the beginning of each experimental session, the temperature-controlled chamber that housed the stimulus pup was warmed to between 35 and 37 °C. A 6- to 8-day-old stimulus pup was then confined inside the chamber with the aid of a secondary plastic mesh tube. The pup habituated to the environmental chamber for 20 min, during which time vocalizations ceased. One anosmic mother and one control mother were then placed in the two observation cages adjacent to the pup and were allowed to habituate to the apparatus for 40 min. At the completion of this habituation period, a time-lapse VCR was started, and a 20-min baseline recording was made. During both the habituation and the baseline periods, the pup remained silent. After the conclusion of the baseline period, the water supply regulating the temperature inside the environmental chamber was

changed, lowering the ambient temperature around the pup to ≈ 15 °C. Soon thereafter, the stimulus pup began to produce USVs. Production of USVs was monitored with the aid of the bat detector, and a 20-min test session was video recorded, beginning with the production of the first USV.

At the end of each experimental session, anosmic and control mothers had been exposed to two different stimuli: a warm silent pup and a cool vocalizing pup. The use of a matched pairs experimental design in which anosmic and control mothers were tested simultaneously during exposure to the same stimulus pup, was crucial to the interpretation of results because stimulus pups do not vocalize at the same rate or intensity. This matched pairs (yoked) experimental design ensured that both control and experimental animals received equivalent USV exposure.

Induction of anosmia. Anosmia was induced using the basic procedure of Alberts and Galef (1971), with modifications described by Alberts and Friedman (1972). Rats were anesthetized with isoflourane (4% in oxygen), and ZnSO₄ solution (7.65%) was perfused through the nasal cavity by way of the posterior choanae, with the aid of a 1-cc syringe attached to a hooked catheter adapted from a blunted 22-gauge needle. ZnSO₄ solution was perfused into the nasal cavity until 9 drops of the solution emerged from the external nares. This typically required the injection of 0.5 to 0.8 cc of solution. Once the solution had been administered, the catheter was removed, and the mouth and throat were aspirated to remove excess solution. Rats were held head down until they recovered from anesthesia, facilitating drainage of the ZnSO₄ solution from the nares. Control rats were perfused with physiological saline.

Verification of anosmia. Traditionally, anosmia is verified by examining the accuracy with which rats can locate buried food (e.g., Alberts, 1974). Unfortunately, this procedure requires both extensive training and food deprivation, both of which presented problems for an experiment that involved testing lactating mothers at specific postpartum stages. By capitalizing on mothers' natural motivation to retrieve pups, we were able to develop a procedure that did not require food deprivation, thereby precluding undesirable side effects that might have accompanied this manipulation. In addition, minimal training requirements made it possible to precisely time tests for responsiveness to a vocalizing pup.

Mothers were tested for their ability to correctly locate buried anesthetized stimulus pups (3 days of age) both before and after the induction of anosmia. The first test was conducted immediately before nasal perfusion (24 hr before testing for responses to USVs), and the second test was performed immediately after the assessment of responsiveness to a vocalizing pup. Testing was conducted in a standard polycarbonate maternity cage (45 × 25 × 20 cm) using an apparatus that consisted of four opaque plastic cups (9 × 9 × 4 cm) attached in series to a piece of sheet aluminum (36.5 × 9 cm). This apparatus was positioned adjacent to the long wall of the testing cage at the beginning of each trial.

Each testing session was composed of a priming phase and a test phase. The priming phase was designed to motivate mothers to retrieve pups from the four cups. At the beginning of each priming trial, an unanesthetized foster pup (3 days old) was placed in each of the four cups. The apparatus was then placed in the cage, and mothers were allowed to retrieve the pups. Priming sessions ended when the mother had retrieved all four pups or 5 min had elapsed. Mothers received a total of four priming trials before testing.

The four priming trials were followed by 10 test trials, during which mothers were allowed to retrieve a buried anesthetized pup from one of the four test cups. Stimulus pups were anesthetized with urethane (1,250 mg/kg ip), eliminating both movement and the production of USVs. At the beginning of each test trial, an anesthetized stimulus pup was placed in one of the four test cups, and all four of the cups were filled with aspen shavings, preventing mothers from seeing the pup. A piece of gauze was subsequently placed on top of each of the four cups, and the apparatus was lowered into the test cage. Test trials ended when the mother had retrieved the buried pup or 5 min had elapsed. Pups were assigned to the four plastic

cups using a pseudorandom procedure (rolling a four-sided die), with the one restriction being that stimulus pups could not be placed in the same cup on four consecutive trials.

During each test trial, an observer recorded the cup that the mother first chose to explore. Mothers were deemed to have chosen a particular cup if they pushed aside the gauze on top of a cup and began to root in the shavings. After the completion of testing, data were converted to a percentage of correct responses. Mothers responded correctly on a given trial if the pup was located in the cup they chose to explore first. Incorrect initial choices, as well as failures to explore any of the cups, were treated as incorrect responses. Before being rendered anosmic, mothers typically retrieved buried pups with 70 to 100% accuracy. One mother was eliminated from the experiment (and not included in above group sizes), however, because she failed to retrieve pups at levels significantly better than chance before the induction of anosmia.

Data analysis. The duration of proximal orientation displayed by each anosmic and control mother during the baseline period was subtracted from the duration during the subsequent test period, yielding difference scores that reflected the change in proximal orientation elicited by stimulus pup vocalizations. Data are presented as mean (\pm SEM) values of these difference scores for each group of anosmic and control mothers. Differences between the mean difference scores obtained from anosmic and control mothers were tested for statistical significance using matched pairs *t* tests, as individual pairs of anosmic and control mothers were exposed to the same vocalizing stimulus pup. Differences between means were considered to be statistically significant if $p < .05$ (two-tailed).

Individual rats were considered to have performed at levels better than those predicted by chance ($p < .05$) in anosmia verification tests if they chose correctly on 7 or more of the 10 test trials. This criterion was arrived at using a binomial distribution with $p = .25$ and $N = 10$.

Results and Discussion

Tests examining retrieval of buried pups provided evidence that the ZnSO₄ treatment effectively induced a profound olfactory deficit (anosmia). On average, mothers in both the saline- and the ZnSO₄-treated groups retrieved buried pups with 80–90% accuracy before nasal perfusion (Figure 4A). All animals used in the present experiment performed at levels better than chance during these initial baseline tests. After perfusion, the behavior of saline-treated mothers was virtually unchanged (Figure 4b). Only a single saline-perfused mother failed to perform at levels better than chance after nasal perfusion. In contrast, ZnSO₄-treated mothers correctly located buried pups on an average of only $3.8 \pm 2.6\%$ of the test trials. None of the ZnSO₄-treated mothers correctly located stimulus pups at better than chance levels. ZnSO₄-treated rats failed to dig in any of the bedding-filled cups during many of the test trials conducted after nasal perfusion. This failure to search cannot be attributed to a general lack of motivation to retrieve: All of the ZnSO₄-treated mothers retrieved exposed pups during priming trials.

As predicted, anosmia attenuated mothers' responses to a vocalizing pup. During the baseline period, ZnSO₄- and saline-treated mothers engaged in proximal orientation for an average of 5.3 ± 2.9 s and 98.3 ± 32.2 s, respectively. During the test period, when the pup was vocalizing, saline-treated mothers displayed an average increase in proximal orientation of 411.8 ± 48.0 s (see Figure 5). Unlike their saline-treated counterparts, anosmic mothers engaged in only 81.0 ± 32.3 s of additional proximal orientation during USV exposure. A paired *t* test comparing the mean increases in proximal orientation displayed by saline- and ZnSO₄-treated mothers during the test period revealed

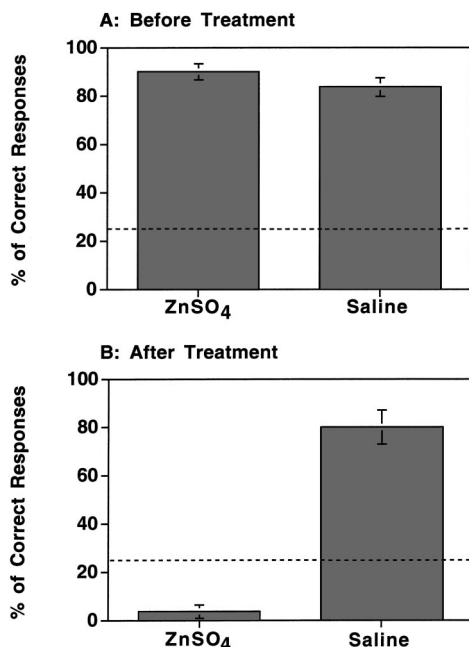


Figure 4. Retrieval of buried pups both before (A) and after (B) treatment with ZnSO₄ or saline. Histograms represent the mean percentage of correct responses on 10 test trials (\pm SEM). Dashed lines represent the percentage of correct responses predicted by chance.

a statistically significant difference between anosmic mothers and controls, $t(7) = 6.72$, $p < .01$.

The reduction in responsiveness to a vocalizing pup after peripherally induced anosmia indicates that under normal circumstances, olfaction co-acts with auditory perception to elicit enhanced maternal responses to USVs. Unlike ZnSO₄-treated mothers, saline-treated mothers displayed dramatic increases in proximal orientation during USV exposure. Because anosmic mothers and saline-treated mothers were tested during simultaneous exposure to the same stimulus pups, the attenuated responses displayed by anosmic mothers cannot be attributed to variability in the acoustic characteristics of vocalizing stimulus pups. The vigorous responses displayed by saline-treated mothers during exposure to vocalizing pups also replicate earlier findings with mothers of 6- to 8-day-old pups (Farrell & Alberts, 2002).

Experiment 3: Responsiveness to USVs: Do Mothers Use Pup Odors as a Directional Cue?

The results of the previous two experiments indicate that maternal responsiveness to a vocalizing pup requires concurrent exposure to acoustic and olfactory stimuli. Mothers and virgins exhibited similar increases in proximal orientation when recorded USVs were presented alone. Only when a warm silent pup was positioned under the playback speaker did mothers exhibit an enhanced response to USVs relative to virgin controls. Furthermore, the potentiated maternal response to USV was eliminated by peripheral anosmia. The results presented thus far are in general agreement with those reported by Smotherman et al. (1974, 1978) indicating that ambient pup odors facilitate maternal approach toward recorded USVs in a Y maze.

Whereas the aforementioned findings indicate that pup odors affect the mother rat's responses to USVs, the kind of effect that these odors exert on the dam remains unclear. Pup odors may provide mothers with directional information that they use when responding to USVs. Alternatively, these odors may serve as a general potentiating stimulus for responsiveness to USVs. That is, pup odors may simply create a context in which mothers are more responsive to USVs and possibly other sounds.

Smotherman et al. (1974) found that when recorded USVs were played from a speaker located at the end of one arm of a Y maze and the other arm was left empty, mothers entered both arms at chance levels. Only when a comatose pup was positioned at the end of the opposing arm of the maze (not visible from the start box) did mothers reliably prefer the arm of the maze containing the ultrasonic speaker. Such results led Smotherman et al. (1974) to conclude that "for the lactating female rat the presence of an olfactory cue from a displaced pup is a necessary condition for retrieval. This cue, however, lacks directional properties" (p. 61). These authors went on to suggest that olfactory "cues initiate the searching behavior to which ultrasounds give direction" (p. 61).

The proposition that olfactory cues serve as a general potentiating stimulus for maternal retrieval is supported by the finding that response latencies were reduced when a comatose pup was positioned in the Y maze (Smotherman et al., 1974). The extent to which mothers use pup odors as directional cues however, remains unclear. In Smotherman et al.'s (1974) experiment, the start box for the Y maze was the mothers' home cage, which contained soiled bedding and, presumably, the offspring of the mother being tested. If pup odors truly lack directional properties, then it would be reasonable to predict that odors emanating from the start box should induce a preference for recorded USVs regardless of whether a comatose pup was positioned on the maze in opposition to the source of recorded USVs.

Experiment 3 was designed to determine whether mothers use nonacoustic elements of the pup stimulus as a directional cue when responding to USVs in our paradigm. To do this, we presented

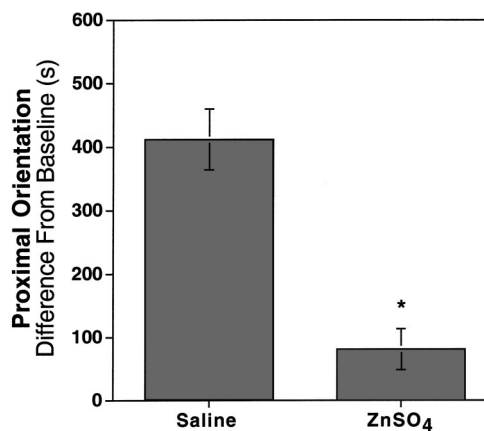


Figure 5. Duration of proximal orientation by anosmic (ZnSO₄-treated) mothers of 6- to 8-day-old pups and saline-treated controls. Histograms represent the mean difference between the 20-min test period (pup vocalizing) and the 20-min baseline period (pup silent). Error bars represent the SEM. Statistical comparisons were made against matched controls (* $p < .05$).

individual mother rats with recorded USVs in the presence of a warm silent pup. For the control mothers, USVs emanated from a speaker positioned just above the warm silent pup. For the remaining mothers, USVs and pup odors were spatially dissociated: The playback speaker was positioned outside one side of the observation cage, and a warm silent pup was positioned outside an equivalent opening cut in the opposing wall of the cage.

Differences in mothers' behavior caused by the position of the warm silent pup should indicate that a directional cue emanates from the pup. Alternatively, if pup odors act as a general nondirectional potentiating stimulus, mothers should orient toward the playback speaker with the same duration in both stimulus configurations.

Method

Subjects. Twenty Sprague-Dawley rat (*Rattus norvegicus*) mothers of 6- to 8-day-old pups served as subjects. In addition, twenty 6- to 8-day-old stimulus pups were used to provide mothers with nonacoustic pup stimuli. Rats were housed under the same conditions described in Experiment 1.

Apparatus. The apparatus used to test behavioral responsiveness to recorded USVs in the presence of nonvocal pup stimuli was the same as that used in Experiment 1, with the exception of two modifications made to the observation cage. First, the observation cage was outfitted with two identical mesh-covered holes (10.2 cm in diameter) positioned on the two opposing walls of the observation cage aligned with the playback speaker. Second, because the semienclosed nesting area used in the previous experiment would have blocked the newly cut hole in the observation cage, this enclosure was replaced with a three-sided, white plastic enclosure (15.5 × 10 × 12 cm) that was centered against the back wall of the observation cage with the open side facing the video camera. The top of the enclosure was solid plastic, and the sides were composed of a plastic lattice (holes measured 0.5 × 2.0 cm) that allowed the passage of sound into the enclosure.

Procedure. Mothers were tested using two different stimulus configurations. Ten mothers were tested for behavioral responsiveness to recorded USVs, while a warm silent pup was positioned directly under the playback speaker. For the remaining 10 mothers, recorded USVs were played from a speaker located outside one side of the observation cage, while the warm silent pup was positioned outside the mesh-covered hole cut in the opposing side of the cage. Recorded USVs and nonvocal pup stimuli were presented from both the left- and right-hand sides of the observation cage in a counterbalanced fashion. Data from one rat (not included in above group sizes) were dropped from the analysis because of excessive levels (>600 s) of proximal orientation directed toward the warm silent pup during the baseline period.

The playback method used was similar to that used in Experiment 1. However, because pilot observations suggested that mothers required additional time to habituate to the reconfigured observation cage, the 40-min habituation period used in previous studies was replaced with a 2-hr habituation period. As in previous studies, the habituation period was followed by a 20-min baseline (silent) period and a 20-min test (USV playback) period.

Data analysis. After the completion of the experiment, video recordings obtained during both the baseline and test periods were coded for the duration of proximal orientation displayed toward each of the two openings in the sides of the observation cage. The duration of proximal orientation displayed by individual animals toward each of the two holes during the baseline period was subtracted from values obtained during the test period. Data are presented as the mean values of these difference scores ($\pm SEM$). Because individual pairs of mothers tested in both stimulus configurations were presented with the same sets of recorded USVs, comparisons between means were conducted using paired *t* tests. Similar comparisons were also

made between the mean durations of proximal orientation displayed toward the openings in both sides of the cage (combined) during the test period for the two different stimulus configurations. Differences between means were considered to be statistically significant if $p < .05$ (two-tailed).

Results and Discussion

Pup odors appear to act as a directional stimulus for maternal responses to USVs in our paradigm. Consistent with the results of Experiment 1, mothers responded vigorously to recorded USVs when a warm silent pup was positioned beneath the playback speaker. During the baseline period, mothers spent an average of 21.6 ± 8.6 s oriented toward the opening on the empty side of the cage and 91.8 ± 34.4 s oriented toward the silent pup and inactive ultrasound speaker. During the test period, proximal orientation toward the speaker and pup increased by an average of 532.8 ± 75.7 s, whereas proximal orientation toward the empty opening decreased by 13.2 ± 8.0 s (see Figure 6A).

Recorded USVs elicited smaller increases in proximal orientation when the warm, silent pup was positioned outside the opening opposite to the playback speaker. Mothers tested using this stimulus configuration spent an average of 65.4 ± 32.9 and 18.6 ± 11.8 s of the baseline period oriented toward the stimulus pup and speaker, respectively. During USV playback, proximal orientation toward the playback speaker increased by an average of 139.8 ± 33.2 s, and proximal orientation toward the pup increased by an average of 83.4 ± 58.7 s (see Figure 6B). A paired *t* test comparing the mean increases in proximal orientation toward the speaker in the two different stimulus configurations revealed a statistically significant difference, $t(9) = 4.36$, $p < .01$, indicating that the

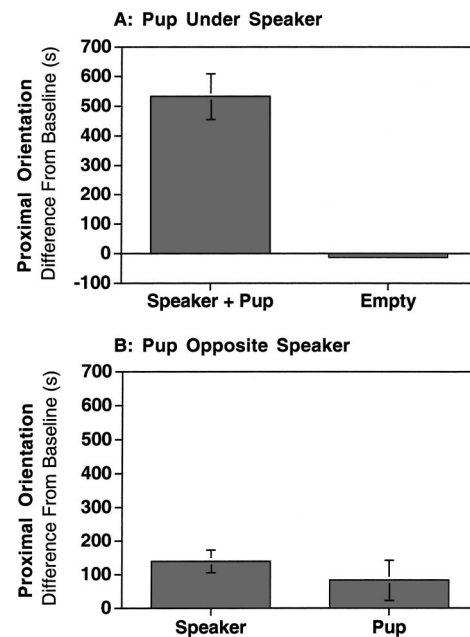


Figure 6. Duration of proximal orientation by mothers of 6- to 8-day-old pups during playback of recorded ultrasonic vocalizations (USVs) when a warm silent pup was positioned either below the playback speaker (A) or outside the opposing mesh-covered hole (B). Histograms represent the mean difference between the 20-min test period (USV playback) and the 20-min baseline (silent) period. Error bars represent the SEM.

position of the warm silent pup influenced responsiveness to recorded USVs. The mean increase in proximal orientation directed toward the warm silent pup positioned across the cage from the playback speaker was not statistically different from proximal orientation toward the empty opening when the pup was positioned beneath the playback speaker, $t(9) = 1.57, p < .15$.

Pup position not only influenced the increased proximal orientation directed toward the playback speaker, it also affected the total duration of proximal orientation displayed during the test period (the sum of proximal orientation directed toward both openings). On average, mothers tested with the pup under the playback speaker and mothers tested with the pup in opposition to the playback speaker displayed 633 ± 89.3 and 307.2 ± 52.13 s of proximal orientation, respectively. The results of a paired t test conducted on the total duration of proximal orientation displayed by mothers during the test period for each of the two stimulus configurations demonstrated that these means were significantly different, $t(9) = 3.03, p < .05$.

Differences in the total duration of proximal orientation during the test period might have resulted from mothers spending more time in transit between the two sides of the cage when the pup was positioned across from the speaker. This, however, does not appear to be the case. Mothers tested with the pup in opposition to the speaker did show more shifts in proximal orientation from one side of the cage to the other ($M = 5.6, SEM = 1.3$) compared with mothers tested with the pup under the speaker ($M = 1.4, SEM = 0.5$). Nevertheless, even if 6 s (more than enough time to traverse the 32-cm length of the cage) is added to the total duration of proximal orientation displayed by each rat for each shift in proximal orientation, as a correction for such transit times, the scores for the two groups remain statistically different ($M = 641.4, SEM = 88.6$, and $M = 340.8, SEM = 58.8$, when the pup was under the speaker or in opposition to the speaker, respectively), $t(9) = 2.72, p < .05$.

Smotherman et al.'s (1974) earlier report that mothers selectively orient toward and approach recorded USVs on a Y maze when an ultrasound speaker and comatose pup were placed at the ends of the opposing arms of the maze suggested that pup odors would serve as a general, nondirectional potentiating stimulus for proximal orientation toward recorded USVs in our paradigm. In combination with the results of the previous playback and anosmia experiments, however, the present results indicate that mothers use odors as directional cues when responding to a vocalizing pup. Although our conclusions differ from those of Smotherman et al. (1974), our findings were not entirely inconsistent with their data. When mothers were forced to choose between recorded USVs in one arm of the Y maze and a live, vocalizing pup in the other arm, Smotherman et al. (1974) found that mothers reliably approached the vocalizing pup. Smotherman et al. (1974) attributed this preference to differences in sound quality. We propose that mothers chose the vocalizing pup over the speaker because the pup provided spatially contiguous olfactory and auditory cues.

It should be noted that the size and configuration of our testing apparatus might have influenced the outcome of the present experiment. Because the sources of the USVs and the pup odors were both in close physical proximity to the mother, it is possible that the directional vectors from both stimuli might have been so strong as to create conflicting response demands. It would be interesting to conduct similar spatial dissociation experiments using a larger

testing arena, in which the directional vectors associated with the olfactory and acoustic stimuli would presumably be diminished. Under these circumstances, odors might act as a general potentiating stimulus for orientation toward the source of USVs or vice versa. A larger arena would also make it possible to systematically vary the degree of spatial discrepancy between the two stimuli to determine the degree of contiguity required for the two stimuli to elicit a maximal response.

General Discussion

In our companion article (Farrell & Alberts, 2002), we examined the behavioral responses of pregnant dams, mothers, and virgin female controls during exposure to a stimulus pup that was either warm and silent or cool and vocalizing. We found that enhanced maternal responsiveness to a vocalizing pup, as measured by increased proximal orientation during USV exposure, emerges around the time of birth, continues to develop during the 1st week postpartum, and declines by the time of weaning (21 days postpartum). The present findings indicate that USVs alone are insufficient to elicit an enhanced maternal response to a vocalizing pup. Rather, pup odors interact with USVs in a directional manner to enhance maternal proximal orientation toward a vocalizing pup.

The results of our initial playback study (Experiment 1) demonstrated that nonacoustic cues are required for an enhanced maternal response to USVs. When recorded USVs were presented alone, mothers and virgins responded with equivalent increases in proximal orientation. Mothers displayed enhanced responses relative to virgin controls only when a warm silent pup was located below the playback speaker. The size and configuration of our playback apparatus, and the limited illumination during testing, led us to conclude that pup odors most likely were the crucial non-acoustic stimulus required for an enhanced maternal response to USVs. The results of Experiment 2 support this conclusion. Maternal responsiveness to a live vocalizing pup was dramatically attenuated after the induction of peripheral anosmia. The findings obtained from the playback experiment involving dissociated vocal and nonvocal pup cues (Experiment 3) suggest that mothers use pup odors as a directional cue when responding to a vocalizing pup. Spatial contiguity between auditory and nonauditory pup cues was required for mothers to display maximal proximal orientation toward the playback speaker.

Beach and Jaynes (1956) suggested that the mother rats' retrieval of displaced pups is under multisensory control. That is, they posited that retrieval can be initiated and directed by various pup cues that impinge on different sensory modalities. Mothers continue to retrieve pups that have strayed from the nest even when one (and sometimes more than one) sensory system has been incapacitated. Whereas Beach and Jaynes focused on the mother rat's retrieval response as a whole, the present study focused on one aspect of maternal responsiveness that may play a role in retrieval, namely, maternal responsiveness to a vocalizing pup. Unlike retrieval, the enhanced maternal response to a vocalizing pup requires concurrent auditory and olfactory stimulation.

One possible explanation for the mother rat's enhanced response to USVs is that the physiological changes associated with pregnancy, birth, and lactation alter the way in which olfactory and auditory inputs are integrated. If this is the case, the mother's enhanced response to a vocalizing pup could result from her

altered instantaneous perceptions of both pup odors and USVs. Although the present data do not specifically indicate that integration of this type is occurring, the possibility cannot be ruled out.

Another, more likely explanation for mother rats' enhanced responsiveness to a vocalizing pup is that USVs enhance the saliency of pup odors. This idea is consistent with Bell's (1974) proposition that USVs produce a state of arousal in adult rats that hear them. Internal, integrative mechanisms may not be required to explain a sequential phenomenon of this nature. Rather, initial behavioral responses to USVs could increase the saliency of pup odors themselves. Pup odors may then cause mothers to display additional proximal orientation while inhibiting virgins from doing the same.

One way in which USVs might increase the perceptual strength of pup odors is simply by bringing the receiver (i.e., a mother or virgin rat) into closer proximity to an odoriferous, vocalizing pup. Mothers and virgin females both have a tendency to approach USVs. When presented with recorded USVs in the absence of pup odors (Experiment 1), mothers and virgins displayed equivalent increases in proximal orientation. When USVs are generated by a live stimulus pup rather than a playback speaker, an initial tendency to orient toward and approach USVs would expose both mothers and virgins to more intense pup odors.

Aspects of mothers' and virgins' responses to USVs, in addition to direct approach to the source of the sound, may also act to increase the saliency of pup odors and thereby contribute to differences in proximal orientation. Welker (1964) reported that nonolfactory sensory stimuli, including tactile and auditory stimulation, reliably elicit bouts of sniffing. Although we did not systematically examine sniffing in our behavioral analysis, both mothers and virgins appear to frequently sniff the air during USV exposure. This sniffing can both precede and accompany the proximal orientation response. Allin and Banks (1972) also describe sniffing during USV playback. Presumably, sniffing in response to USVs would increase the saliency of pup odors by increasing airflow over the nasal epithelium.

A sequential response to USVs and pup odors, such as the one outlined above, can only explain our findings if the adult female rats' perception of pup odors changes over the course of the maternal behavior cycle in such a way that mothers find these odors more attractive than do virgins. Given both the present data and those reported by other researchers, this seems to be a plausible assumption.

In addition to suggesting that hormones render mothers less timid than virgins, Fleming (1986) has proposed that hormonal changes associated with the transition from pregnancy to motherhood make pup odors attractive to mothers. Latencies to become maternally responsive after long-term exposure to foster pups are greatly reduced in anosmic virgins relative to intact controls, suggesting that pup-naive virgin females find pup odors aversive (Fleming & Rosenblatt, 1974a, 1974b; see also Kinsley & Bridges, 1990). Such an aversion might curtail proximal orientation to a vocalizing pup by virgin females. The proposition that endocrine changes associated with pregnancy render pup odors attractive is supported by the finding of Fleming et al. (1989) that steroid-treated virgins tested on an open field spend more time in close proximity to shavings obtained from the cage of a lactating dam than do untreated virgin controls. Bauer (1983) has also reported that preferences for nest odors change during the course of the

maternal behavior cycle. Pregnant dams begin to show a preference for the odor of soiled shavings obtained from their own nest or the nest of another lactating dam (comparison against clean shavings) shortly before giving birth. This preference increases during the 1st week postpartum and declines during the following week. Kinsley and Bridges (1990) also reported that pregnant dams exhibit a preference for pup-related odors (vs. clean shavings) in a T maze when tested shortly before giving birth (Gestational Day 22).

Although pup odors are presumably a significant olfactory component in the shavings obtained from the nests of lactating dams, Bauer (1983), Fleming et al. (1989), and Kinsley and Bridges (1990) did not directly test preferences for pup odors. Our findings, however, suggest that mothers are specifically attracted to pup odors. During the tests conducted to verify ZnSO₄-induced anosmia, the intact mothers of 6- to 8-day-old pups correctly located and retrieved buried, anesthetized stimulus pups with far better accuracy than would be predicted by chance. Pup odors were the only cue available to elicit and direct this response.

If USVs evoke from mothers and virgins behavioral responses that increase the saliency of odors presented contiguous with these sounds, then changes in the perception of pup-related odors during the course of the maternal behavior might explain the majority of our prior and current findings. Steroid-induced increases in the attractiveness of pup odors could explain why steroid-treated virgins and mothers of newborns respond to vocalizing pups with increases in proximal orientation and control virgins do not (Farrell & Alberts, 2002). In addition, further postpartum changes in the perception of pup-related odors, such as those reported by Bauer (1983), may also explain the potentiation of maternal responsiveness to a vocalizing pup during the 1st week after birth and the subsequent decline in this response by weaning (Farrell & Alberts, 2002). The results of the present playback and anosmia studies are also consistent with an explanation based on increased maternal preferences for pup odors. Taken together, our present and previous results (Farrell & Alberts, 2002) suggest that maternal responsiveness to USVs alone is not a maternal specialization in the Norway rat, though responsiveness to the complex stimulus of a vocalizing pup may be such a specialization.

References

- Alberts, J. R. (1974). Producing and interpreting experimental olfactory deficits. *Physiology and Behavior*, *12*, 657-670.
- Alberts, J. R., & Friedman, M. I. (1972, August 25). Olfactory bulb removal but not anosmia increases emotionality and mouse killing. *Nature*, *238*, 454-455.
- Alberts, J. R., & Galef, B. G. (1971). Acute anosmia in the rat: A behavioral test of a peripherally-induced olfactory deficit. *Physiology and Behavior*, *6*, 619-621.
- Allin, J. T., & Banks, E. M. (1972). Functional aspects of ultrasound production in infant albino rats (*Rattus norvegicus*). *Animal Behaviour*, *20*, 175-185.
- Bauer, J. H. (1983). Effects of maternal state on the responsiveness to nest odors of hooded rats. *Physiology and Behavior*, *30*, 229-232.
- Beach, F. A., & Jaynes, J. (1956). Studies of maternal retrieving in rats. III. Sensory cues involved in the lactating female's response to her young. *Behaviour*, *10*, 104-125.
- Bell, R. W. (1974). Ultrasounds in small rodents: Arousal-produced and arousal-producing. *Developmental Psychobiology*, *7*, 39-42.
- Brown, A. M. (1973). High levels of responsiveness from the inferior

- colliculus of rodents at ultrasonic frequencies. *Journal of Comparative Physiology*, 83, 393–406.
- Crowley, D. E., Hepp-Reymond, M.-C., Tabowitz, D., & Palin, J. (1965). Cochlear potentials in the albino rat. *Journal of Auditory Research*, 5, 307–316.
- Farrell, W. J., & Alberts, J. R. (2002). Maternal responsiveness to infant Norway rat (*Rattus norvegicus*) ultrasonic vocalizations during the maternal behavior cycle and after steroid and experiential induction regimens. *Journal of Comparative Psychology*, 116, 286–296.
- Fleming, A. S. (1986). Psychobiology of rat maternal behavior: How and where hormones act to promote maternal behavior at parturition. *Annals of the New York Academy of Sciences*, 474, 234–251.
- Fleming, A. S., Cheung, U., Myhal, N., & Kessler, Z. (1989). Effects of maternal hormones on “timidity” and attraction to pup-related odors in female rats. *Physiology and Behavior*, 46, 449–453.
- Fleming, A. S., & Rosenblatt, J. S. (1974a). Olfactory regulation of maternal behavior in rats: I. Effects of olfactory bulb removal in experienced and inexperienced lactating and cycling females. *Journal of Comparative and Physiological Psychology*, 86, 221–232.
- Fleming, A. S., & Rosenblatt, J. S. (1974b). Olfactory regulation of maternal behavior in rats: II. Effects of peripherally induced anosmia and lesions of the lateral olfactory tract in pup-induced virgins. *Journal of Comparative and Physiological Psychology*, 86, 233–246.
- Hofer, M. A., & Shair, H. N. (1980). Sensory processes in the control of isolation-induced ultrasonic vocalizations by 2-week-old rats. *Journal of Comparative and Physiological Psychology*, 94, 271–279.
- Hofer, M. A., & Shair, H. N. (1987). Isolation distress in two-week-old rats: Influence of home cage, social companions, and prior experience with littermates. *Developmental Psychobiology*, 20, 465–476.
- Hofer, M. A., & Shair, H. N. (1991). Trigeminal and olfactory pathways mediating isolation distress and companion comfort responses in rat pups. *Behavioral Neuroscience*, 105, 699–706.
- Kinsley, C. H., & Bridges, R. S. (1990). Morphine treatment and reproductive condition alter olfactory preferences for pup and adult male odors in female rats. *Developmental Psychobiology*, 23, 331–347.
- Rosenblatt, J. S., & Lehrman, D. S. (1963). Maternal behavior of the laboratory rat. In H. L. Rheingold (Ed.), *Maternal behavior in mammals* (pp. 8–57). New York: Wiley.
- Smotherman, W. P., Bell, R. W., Hershberger, W. A., & Coover, G. D. (1978). Orientation to rat pup cues: Effects of maternal experiential history. *Animal Behaviour*, 26, 265–273.
- Smotherman, W. P., Bell, R. W., Starzec, J., Elias, J., & Zachman, T. A. (1974). Maternal responses to infant vocalizations and olfactory cues in rats and mice. *Behavioral Biology*, 12, 55–66.
- Thiels, E., Alberts, J. R., & Cramer, C. P. (1990). Weaning in rats: II. Pup behavior patterns. *Developmental Psychobiology*, 23, 495–510.
- Welker, W. I. (1964). Analysis of sniffing in the albino rat. *Behaviour*, 22, 223–244.

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