The Ontogeny of Maternal Potentiation of the Infant Rats’ Isolation Call

ABSTRACT: Previous studies have shown that preweanling rat pups double or triple their rates of ultrasonic vocalization (USV) when isolated immediately after brief periods of maternal interaction (potentiation). We studied the ontogenetic pattern of USV and other behavioral responses of pups to 3-min periods of isolation in a novel test chamber, from 5 to 25 days postnatal age, before, during, and after 1 min or 5 min of interaction with an anesthetized or an active dam. USV potentiation did not develop until 7–9 days postnatal, a week after the initial isolation and maternal contact quieting responses were well established. Potentiation reached a peak at 13 days, and then declined until all USV responses ceased after 21 days. Other behavioral responses to isolation were not enhanced by maternal interaction at any age. The distinct ontogenetic pattern of this unusual response to maternal separation has implications for understanding its mode of development and possible adaptive value. © 1998 John Wiley & Sons, Inc. Dev Psychobiol 33: 189–201, 1998.

Keywords: ultrasonic vocalization; rat pups; development; potentiation; maternal separation; isolation distress; attachment

INTRODUCTION

Since the earliest description of infant rodent ultrasonic vocalizations (USVs) by Zippellius and Schleidt (1956), numerous studies of its eliciting conditions and modulating influences (reviewed in Hofer, 1996) have shown that the highest rates of calling are elicited by separating the pup from littermates and home cage nest and placing it alone in novel surroundings at cool temperatures. These isolation calls, together with pup odor, function to elicit and direct maternal search, pup carrying, and retrieval (Smotherman, Bell, Starzac, & Elias, 1974). Pups also vocalize in other contexts. When the home nest is uncovered or disrupted by flooding and the dam is disturbed, high levels of pup USV increase the likelihood of maternal transport of the litter to another location (Brewster & Leon, 1980). Similarly in the laboratory, when pups are repeatedly taken from the nest for testing, the dam becomes disturbed and carries the remaining pups about the cage, scattering the litter group. For this reason, dams are generally removed from the home cage a few minutes prior to testing the pups’ isolation response. For the
isolated pup, contact with the dam, with littermates, or with home cage shavings markedly reduces calling rates (Hofer & Shair, 1987; Oswalt & Meier, 1975). During the naturally occurring nursing cycle, pups are generally silent except for low levels of USV that are limited to the first minute or two following the arrival and the departure of the dam (Hofer & Shair, 1978). Thus, maternal contact has been thought to have a primary quieting effect on pups, while pup USV serves to arouse the dam and direct particular maternal activities. These characteristics are typical of mammalian mother–infant vocal communication systems, although isolated primate infants have been reported to increase calling rates when their dam is perceived to be nearby, but in a different cage, an effect that has been interpreted as a coping strategy (Boyart, Hayashi, Faull, Barchas, & Levine, 1990).

In the course of experiments showing that thermal warming was not necessary for maternal quieting of 12-day-old pups (Hofer, Brunelli, & Shair, 1993), we made the unexpected discovery that after a 3-min period of contact with an anesthetized dam in the test chamber, there occurred a doubling of the pup’s isolation calling rates, regardless of whether the dam’s body had been cooled below ambient temperature or remained warm. Brief contact and removal of anesthetized littermates from the isolated pup produced identical quieting during contact, but the pup’s USV rate merely returned to its original isolation level following removal of the littermates (Hofer, Brunelli, & Shair, 1994). Thus, the enhanced isolation response was specific to maternal cues and not simply a rebound following brief suppression, or a response to sudden thermal contrast. We called this effect “maternal potentiation,” because the combined action of brief maternal contact and isolation produced a far greater USV response than the sum of the effects of each alone. We next showed that brief contact was just as effective if it took place outside of the test chamber, in the home cage nest, and that repeated maternal separations did not produce higher USV rates than the first separation (Hofer, Brunelli, Masmela, & Shair, 1996). Nor was it simply the act of separating the pup directly from its dam that enhanced the pups’ USV response, because pups remaining with anesthetized dams for longer time periods (30 min) prior to isolation showed no evidence of potentiation, even when milk ejections and anogenital stimulation were provided experimentally during this interval. By measuring the other behaviors of the isolated pups, we determined that potentiation was limited to the pups’ vocalizations, rather than being part of a generalized arousing response: other behaviors elicited by isolation at this age (locomotion, rising, and face washing) remained unchanged or were reduced following brief maternal contact (Hofer et al., 1994; Hofer et al., 1996).

The possibility remained that potentiation was a unique effect of the unresponsiveness of the anesthetized dam. Instead, we found that active (unanesthetized) dams were even more effective in potentiating subsequent USV responses to isolation in 12-day-old pups, tripling or quadrupling the USV rates elicited by standard isolation testing (Hofer et al., 1996). Short periods of active interaction were not characterized by the prolonged body contact seen with anesthetized dams. Instead, instances of maternal retrieval, nosing, body licking, partial breaks in contact, and maternal repositioning of pups were observed, with few of the pups being nipple-attached at the time of separation. These results clearly disproved that it was the contact and quieting action of the anesthetized dam that was responsible for the enhanced response of pups to their subsequent isolation. Indeed, periods of active maternal interaction continued to be effective, even when prolonged to 30 min.

These findings provided a working description of maternal potentiation as it occurred in pups toward the end of their 2nd postnatal week, the age at which it was discovered. In order to better understand this novel effect, we needed to know its ontogenetic pattern. Did it first occur at 2–3 days postnatal age, together with isolation distress vocalization and maternal contact quieting or did it develop later, after these more familiar responses were well established? An alternative developmental course would suggest that maternal potentiation originated in different developmental processes and played a different adaptive role in the changing ecology of the infant rat than do the other two better-known vocal responses. For example, might maternal potentiation extend the age range during which pups continue to vocalize in response to isolation, beyond the early weaning period (18–20 days) when the isolation distress response is known to come to an end? In addition, we wondered whether potentiation responses to active and to passive dams first appear together at the same postnatal age or whether one precedes the other. Was active potentiation more effective than the passive form at all ages, or just in the age period we have studied thus far (11–13 days)? Here again, different ontogenies would tend to differentiate these two forms of the potentiation response. At 12 days of age, duration of maternal exposure had been found to be important for passive, but not for active potentiation (Hofer et al., 1996). Would temporal factors be important at other ages and when would each of these responses be maximal? Finally,
although at 12 days of age we did not find any evidence that USV potentiation was part of a heightened level of general behavioral activation, this might not be the case at other stages in the development of the potentiation effect, either when it first appeared or when it was maximally expressed.

Thus we set out to test pups for maternal potentiation of the isolation response at a series of ages from the 1st to the 3rd postnatal weeks, evaluating the effects of 1-min or 5-min periods of interaction with active or with passive dams. In the course of studying these parameters of USV potentiation, we would simultaneously be observing the ontogenies of the standard isolation distress and maternal contact quieting responses. This developmental approach can allow one to differentiate several responses or physiological processes that may be difficult or impossible to separate from one another when studied at a single age. Furthermore, the different ecological niches that pups occupy at different postnatal ages (Alberts & Cramer, 1988) can provide clues to the developmental processes responsible for the origin of responses arising at particular ages and to the possible survival value of responses that are fully expressed at one age or that decline rapidly at another stage in the pups’ changing postnatal world.

METHODS

Subjects

Rats of a Wistar-derived strain (Hilltop Farms, Scottsdale, PA) that were born and raised in our laboratory were housed in plastic terraria (40 × 40 × 20 cm) with 4–5 cm of wood shavings, under conditions of regulated temperature and humidity. All litters were culled to 9 pups within 72 hr of birth and housed under a 12:12 hr light:dark reversed day–night cycle. Except for routine feeding and watering, the litters were left undisturbed until testing at one of the following ages: 5, 7, 9, 11, 13, 17, and 23 (21–25) postnatal days.

Ultrasound Detection and Rate Measurement. A capacitive microphone with Mylar film diaphragm and preamplifier was used with an ultrasound detector (UltraSound Advice, Ltd., London, Model S-25) which, in the “broadband” mode, transduces sounds between 10 and 100 kHz into the audible frequency range. Further details on this method have been described previously (Hofer & Shair, 1992). Earphones were used to avoid feedback effects to the pups, and ultrasonic calls were counted by pressing a button that activated a silent electronic counter. Reliability tests have shown an interobserver correlation of .97 with this method (Hofer & Shair, 1978).

Test Procedures

On the day of the experiment, between 10 a.m. and 2 p.m., the dam was removed from the home cage and the litter left undisturbed in their home cage nest until testing was begun 20 min later. A thermostatically controlled heat source was placed under the home cage floor to maintain pup temperature at normal nest levels (35–36°C), during the period prior to testing. The dam was placed in her own cage in an adjoining room. Later in the testing, she was anesthetized with urethane (2 g/kg), her nipple lines taped to prevent pup attachment, and then placed in the testing room on a thermoregulated heat source to maintain her body temperature at 37°C between tests. Pups were isolated for 3 min in a polypropylene test chamber (15 × 25 cm) kept at room temperature (22–24°C). Pups were randomly distributed by sex across groups. The USV detector microphone was suspended 5 cm above the center of the test chamber, which rested on an activity platform utilizing electromagnetic field sensors that transduced the field disturbances caused by the pups’ movements into counts/min (Stoelting Co., Wood Dale, IL). The number of ultrasonic calls, activity counts, and four behaviors [squares crossed, pivoting (turns within a square), rises (raising of head and trunk with at least one forefoot off the floor), and self-grooming] were recorded for each minute on time-based data sheets and then placed into an electronic database for computer analysis.

During the periods of dam exposure, USV rate and time out of contact with the dam were recorded. In addition, for active dams, their rates of licking, carrying, and sniffing the pups were recorded. For passive dams, the rates of two pup behaviors, burrowing into and sniffing the dam, were recorded.

The sequence of events that we used to test for maternal potentiation was the same at every age. It consisted of an initial isolation period of 3 min, followed by either 1 min or 5 min of exposure to the dam, and finally, a second 3-min isolation period during which any changes in behavior following maternal interaction could be observed by comparison with the initial isolation period (see diagram at top of Figure 1). To begin each sequence, the pup was picked up by the experimenter from the home cage litter group and placed alone in the test chamber (the standard isolation test). This first 3-min test period was identical for all groups. For the active dam group, the pup was then...
FIGURE 1 USV responses of pups during the test sequence in the 1st postnatal week. The diagram at top left summarizes the three periods of the sequence for 1 min maternal exposure (upper sequence) and 5-min maternal exposure (lower sequence). Within the coordinates, the median USV rate of all four experimental groups ($N = 24$) on their initial (standard) isolation test is represented at each age by the hatched bar at the left and by the hatched line extending horizontally, as a baseline for comparison with the subsequent periods. The median changes from that initial baseline for each group ($n = 6$) are represented by the solid and open bars. The durations of isolation and of dam exposure are indicated by the length of the bars on the time line, with the 1-min and 5-min active dam groups represented by the solid bars and the 1-min and 5-min passive dam groups by open bars. The significance of median changes from the initial isolation period (hatched bar and line) are represented by * and by two-tailed Wilcoxon paired sample ranks test. (‘’) represents a value that is significantly different only in comparison to its matched control group.

picked up, transported to the dam’s cage and placed in contact with her. For the passive dam group, the anesthetized dam was transported to the test chamber where she was placed ventrum down and the pup was placed in contact with her. At the end of either 1 min or 5 min with either dam, the pup was either removed from the active dam’s cage, and then returned to the test chamber alone, or picked up while the anesthetized dam was removed from the test chamber and then left alone in the test chamber. The last 3-min isolation period, like the first, was identical for all experimental and control groups. The pups’ rectal temperatures were taken at the end of testing before returning them to the home cage.

To look for any consistent changes in pup behavior between the first and second isolation periods that might be elicited simply by the manipulation and transport of the pup necessary for exposure to the dam, we tested four control groups, each matched to one of the four experimental groups. In two of these (passive dam controls), the pups were picked up and placed down again in the test chamber at the beginning and end of the 1 min or 5 min between the first and second isolation tests. In the two other groups, the pups were transported to a novel cage (the floor of which was warmed to match the floor of the active dam’s cage) for the 1 min or 5 min between the two isolation periods. In the vast majority of control groups (24/28) there was no significant change in USV rate between first and second isolation tests.

Pups in all eight groups were thus housed together in the home cage under identical conditions throughout the testing session, and each pup was tested under identical conditions in the first and second isolation periods. The order of experimental and control sequences was randomized across repetitions of the experiment at each age, except that exposures to the anesthetized dam always followed those with the dam in the active state, for obvious reasons. Because the intensity of the passive maternal potentiation effect has been shown to be stable over 2-hr periods, in 12-day-olds, (Hofer et al., 1994) and over 1-hr periods in 8-day-old pups (Shair, Masmela, Brunelli, & Hofer, 1997), we are reasonably certain that this fixed aspect of the test order did not systematically affect comparisons between active and passive dam groups. At each age, six litters were tested in this way, with all eight conditions represented in each litter. Males and fe-
males were distributed across the groups in approximately equal numbers.

**Data Analysis**

The experimental design allowed us to make several planned comparisons at each age of testing. We assessed the effects of brief maternal exposure on the pup’s subsequent isolation response by within pup analysis to control for the within subject change score from the first to the second isolation period. A significant increase in USV rate over the pup’s initial isolation level was taken as primary evidence for maternal potentiation. We also assessed the change in USV rate during the pup’s interaction with its dam by a similar within subject change score from first isolation to dam exposure periods. A significant decrease in USV rates from the pup’s initial isolation level defined maternal quieting. Because the matched control groups that were isolated throughout generally showed no significant changes in the first (23/28) or second (24/28) test periods following their initial isolation, this data is not presented except in the few instances of significant change, when comparisons with experimental groups are made in the text. Data from males and females in each group were combined because no significant sex differences were found in initial USV isolation rates or in potentiated isolation rates at any age, or even in pooled samples from groups showing similar responses.

Because of the skewed variance in the USV measure and the small number of pups in each experimental and control group (n = 6), medians instead of means were used, and the significance of changes and differences were assessed by the nonparametric Wilcoxon paired sample signed-ranks test. A conservative, two-tailed, criterion of significance was used with both \( p < .05 \) and \( p < .1 \) levels noted. For the other behavioral measures and for rectal temperatures, means and paired sample \( t \) tests were used.

**RESULTS AND DISCUSSION**

**First Postnatal Week: Isolation Calling and Maternal Quieting Without Potentiation**

This early postnatal period was characterized by high rates of pup USV in response to isolation in the novel test chamber and an equally intense inhibition of USV rates when pups were exposed to their dams. In the second isolation period, when potentiation could be expected to occur following interaction with their dams, no significant increase over initial isolation rates was observed in any group. Instead, some groups showed reduced USV responses to isolation following maternal exposure.

**Five-day-old pups** were the youngest studied. All pups showed a USV response to the initial standard isolation test, ranging from 4 to 137 USV/min with a median of 44 USV/min. Pups in all experimental groups reduced their USV rates in the next period, while interacting with active or passive dams (see Figure 1). But at this early age, pups in three of the four control groups (data not shown) also consistently decreased USV rates this second time period, Wilcoxon paired sample ranks test, \( p < .05 \), so that only during 1 min of contact with the passive dam did pups show a significant further reduction in comparison to their control group.

In the isolation period following maternal contact, pups previously exposed for 1 min or 5 min to the passive dam showed significant reductions in USV rates in comparison to their initial isolation periods (see Figure 1). Thus, instead of potentiation, pups at this early age showed a continuation of the quieting effect of passive maternal contact into the subsequent period of isolation. Pups isolated after interacting with active dams and all four control groups (data not shown) had no significant changes in USV rates between the first and second isolation periods.

The absence of potentiation in the active dam groups at this age cannot be ascribed to a lack of maternal interaction with the pups, because levels of dam carrying and pup licking occurred at levels that were comparable to those at other ages (see Table 1). The persistence of quieting into the second isolation period, which occurred only in the two passive dam groups, cannot be attributed to unusually high rates of dam contact at this age for pups tended to be more out of contact than older pups (Table 1). Nor could it have been due to a warming effect of passive maternal contact, because even the pups in the 5-min passive dam group had rectal temperatures at the end of testing (mean of 32.0°C) that were no greater than their controls (31.9°C and 32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams. This was not a large effect, however, and the 5-min active dam group (32.2°C) at the test’s end, presumably due to having been carried about and licked by their dams.
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to their controls, \( p < .05 \), except in the 5-min active dam group. Consistent with this quieting effect was the measure for pup burrowing under the maternal ventrum of passive dams, which was significantly increased over 5-day-olds (see Table 1).

In the second isolation period, following maternal interaction, none of the groups showed any significant change in USV from their initial isolation rates. However, rates of the 5-min active dam group rose somewhat in their second isolation period, while their control group tended to decrease, \( p < .1 \), leading to a significant difference between experimental and control pups for this group, \( p < .05 \). While this does not meet our definition of potentiation, it does indicate that at this age, 5 min of active maternal interaction can elevate subsequent USV rates in isolation over those expected to occur in pups receiving comparable handling and transport over the same time interval. The other control groups either showed no significant changes in USV rates or mixed responses, the 1-min transport group increasing 14 USV/min, and the 1-min pass group increasing 14 USV/min, \( p < .05 \), data not shown.

Rectal temperatures of pups at the end of the tests were no different from matched controls, except for the 5 min passive dam group (mean = 32.4°C) which tended to be higher than their controls, 31.9°C; \( t = 3.62, df = 5, p = .025 \). Tends to be greater than on day 5, \( t = 2.27, df = 5, p = .07 \). Tends to be greater than on 4 previous days, \( F = 2.73 (4/20), p = .06 \). "greater than 4 previous days, \( F = 6.38 (4/20), p = .002 \.

### Table 1. Measures of behaviors that active dams directed toward pups (top) and that pups directed toward passive dams (bottom) during 1-min and 5-min periods, at different postnatal ages. (Contact is given as mean number per minute, \( \pm \) SEM, and all other behaviors in mean number observed/minute of observation, \( \pm \) SEM.)

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The primary feature of USV responses between postnatal Days 9 and 13 is the gradual appearance of maternal potentiation of the pups' USV response to isolation, with groups exposed to active maternal interaction developing this response at an earlier age than those exposed to passive dams. A significant potentiation effect appeared first in 9-day-olds, in the group with the longer period (5 min) of active maternal interaction prior to isolation. By 11 days of age, both active maternal interaction groups showed powerful potentiation effects, with five- to sixfold increases in isolation-induced USV rates. At this age, the first significant potentiation occurred following contact with a passive dam, but it was a less-powerful effect. Fi-
nally, at 13 days of age, all maternal interaction groups were effective, with the 1-min active dam group showing the most powerful effect.

Nine-day-old pups showed much lower USV responses to their initial isolation than the 7-day-olds, a median of 30 USV/min with a range of 2 to 136 USV/min. All groups showed significant maternal quieting, both in relation to initial isolation rates and to their controls, $p < .05$, except the group with active dams for 5 min (see Figure 2).

In the second isolation period, we saw the first clear-cut potentiation, occurring in the group that had interacted for 5 min with an active dam. This group also increased USV rates significantly above their matched controls, $p < .05$. The groups with 1 min of maternal interaction tended to increase USV rates for the first time at this age, but no other groups showed significant changes from initial isolation levels or in relation to their controls. None of the control groups showed significant changes.

The emergence of potentiation in the active dam groups at this age was not associated with new developments in the maternal interactional measures: time spent out of contact with the dam or in the levels of maternal behavior directed at pups (see Table 1). The experimental pups’ rectal temperatures at this age (range = 31.8–36.1°C) were generally no different from control pups (32.0–36.1°C), except for the 5-min active dam group. This group, the one that showed the earliest evidence of potentiation, had a lower mean rectal temperature (33.9°C) than their controls, 35.5°C; $t = 3.47$, df = 5, $p < .02$, but they did not differ from the 5-min passive dam group (34.0°C) that failed to show any sign of potentiation.

Eleven-day-old pups showed initial USV responses to isolation ranging from 1 to 96 USV/min with a median of 15 USV/min. In a new development at this age, the maternal quieting response became considerably less evident, only the 5-min passive group showing a trend in this direction, $p < .1$.

Potentiation occurred in three of the four dam-exposed groups at this age, with a greater effect in the active than in the passive dam groups, $p < .05$. Pups showed five- to sixfold increases in median USV rates over the standard isolation response after interaction with an active dam. The pups exposed for 5 min to the passive dam lagged behind the others, their second isolation period rates not significantly elevated over initial isolation rates or over their controls during this period. Control groups showed no significant changes over time.

Again, there were no new developments in the levels of behaviors of dams and pups during their periods of interaction at this age (see Table 1). Rectal temperatures at the end of testing showed no differences between experimental groups (range = 33.7–36.3°C) and controls (33.7–35.6°C). The 1-min active dam group (mean = 35.1°C) tended to have higher posttest rectal temperatures than the 1-min passive dam group, 34.7°C; $t = 2.26$, df = 5, $p = .07$.

Thirteen-day-old pups showed a median USV rate of 8/min during their initial isolation period with a median response rate of 90 USV/min, standard deviation of 32 USV/min. The 5-min exposure groups showed significant increases over their initial rates, with the 5-min active dam showing the largest effect, $p < .05$. The 1-min active dam group showed a trend in this direction, $p < .1$.

**FIGURE 2** USV responses of pups in the 2nd postnatal week. The diagram at top left summarizes the three periods of the sequence, and the bars represent the four experimental groups as described in the legend for Figure 1 (solid = active dam, open = passive dam groups). Significance of median changes from the initial isolation period (hatched bar and line) by two-tailed Wilcoxon paired samples ranks test, *$p < .05$, $p < .1$.**
range of 0 to 63/min. None of the groups significantly reduced USV rates during their periods of interaction with their dams, but all of them showed robust potentiation of their USV isolation responses (see Figure 2). Active and passive dam exposures were, for the first time, equally effective as potentiating stimuli, in the 5-min groups. Control pups at this age tended to increase their USV rates from first to second isolation periods, but only in the pick-up controls for the passive dam groups did this reach significance, +15 and +24 USV/min for 1-min and 5-min groups, \( p < .05 \), and in both instances, the dam exposure groups significantly exceeded these control groups, \( p < .05 \). There were no significant changes in dam–pup interaction measures. Rectal temperatures at the end of testing showed no differences between experimental groups (range = 34.1–36.4°C) and controls (34.4–36.2°C) pups. Active and passive dam groups did not differ.

**Third–Fourth Postnatal Week: The Waning of Maternal Potentiation and of Isolation Calling**

As expected from previous studies (Graham & Letz, 1979; Noirot, 1958), initial isolation calling rates declined and then virtually ceased in pups as they reached the age of weaning. All forms of maternal interaction tested continued to potentiate the USV rates of isolated pups early in the weaning period, but with reduced peak rates, and at 23–25 days of age maternal potentiation was no longer evident. Seventeen-day-olds showed a slight lowering of initial isolation calling rates from levels at 13 days of age, to a median of 6 USV/min, with a range from 0–45/min. One quarter of the pups produced no USV during their first isolation, but only one of the 24 dam-exposed pups continued to be silent throughout the potentiation sequence. Only the 5-min passive dam group showed a contact quieting effect, \( p < .1 \) (see Figure 3).

All groups showed significant potentiation following exposure to their dam, with 5 of the 6 pups that were silent in the initial isolation period now calling at rates up to 12/min. USV rates reached during potentiation were only half as high as those at 13 days, but the effect continued to be highly consistent and powerful in relation to initial isolation rates, showing fourfold increases, comparable to younger pups with higher initial rates at the height of the potentiation effect. Control groups showed no consistent changes in USV rates over their sequence of tests.

As at previous ages, there were no major changes in maternal interaction measures at this age (see Table 1). There were no consistent differences in mean rectal temperatures between experimental groups (range = 36.1–37.3°C) and controls (36.1–37.6°C) except for the

**FIGURE 3** USV responses of pups in the 3rd postnatal week. The diagram at top left summarizes the 3 periods of the sequence as in previous figures. Bars represent the four experimental groups as described in the legend for Figure 1 (solid = active dam; open = passive dam groups). Significance of median changes from the initial isolation period (hatched bar and line) by two-tailed Wilcoxon paired samples ranks test, \( p < .05 \). For the 23-day-old group medians, small circles above the bars represent the 2 individual pups with the highest USV rates in either the active dam (filled circles) or passive dam (open circles) groups.
5-min passive dam group (mean = 36.3) which tended to be lower than their controls (36.5); t = 2.44, df = 5, p = .06. No consistent differences were found between active and passive dam groups.

Twenty-three- (21–25) day-old pups vocalized at very low rates, if at all, throughout the test sequence (see Figure 3). Even among those pups that showed some vocal response on their initial isolation (6/24), only 2 showed increased rates when isolated after maternal interaction. Of the 18 pups that were silent during the initial isolation period, only 1 vocalized at all following maternal interaction (10 USV/min). The control groups showed comparably low USV rates throughout. Thus, there was no evidence of the potentiation effect, even in younger pups that continued to vocalize in response to isolation at this age.

There was an increase in out-of-contact times during maternal interactions at this age in the 1 min active dam and the 5-min passive dam groups, reaching levels higher than 9- to 17-day-olds and comparable to the youngest group studied (see Table 1). But these decreased contact times are unlikely to be related to the loss of potentiation at this age because the other two groups (1-min passive and 5-min active) did not differ on this measure from 17-day-old pups that showed consistent potentiation. The other new development in dam behavior at this age is that no pup carrying was observed, even when pups were with the active dam for 5 min. This is consistent with the literature on the ontogeny of this interaction (Brewster & Leon, 1980) and might have contributed to the ineffectiveness of the active dams in potentiating pups’ isolation responses at this age because we have recently found that inducing the pup transport response potentiates the pup’s subsequent USV response to isolation (Hofer, Brunelli, Masmela, and Shair, in press).

There were no consistent differences in mean rectal temperatures between experimental groups (range = 36.3–38.6°C) and controls (36.2–38.5°C).

Changes in Other Isolation-Induced Behaviors

The results were simple and clear-cut: None of the experimental groups, at any age, showed significant increases in either the automated measure of general activity or in any of the individual behaviors elicited by isolation following maternal interaction. The data for the group showing the earliest and most-intense potentiation, the 5-min active dam group, are presented in Figure 4. The trend in every measure at every age was for a decrease in the levels of behaviors (other than USV) from the first to the second isolation period, although group changes in any one measure were not often statistically significant. The littermate control groups showed similar consistent decreases in activity levels.

Individual behavioral responses increased slowly as the pups matured (bottom half of Figure 4). At the first two postnatal ages (5 and 7 days), pups showed only brief trunk, limb, and head movements that were detected as activity counts on the electronic motion detector. Individual behavioral acts began to be expressed at the end of the 1st week, with pivoting, an early form of locomotion, then early rearing (one paw raised against the chamber wall) at 9 days, along with the first forward locomotion sufficient to cross into a new square (8 cm on a side). By 11 postnatal days, face-washing bouts began to occur regularly, and at 13 days, locomotion and rearing began to occur more than once per minute. Weanling age pups moved...
swiftly, locomoting and rising at high rates of 7–12/ min in their first isolation period, with these behaviors rapidly diminishing in their second isolation, presumably due to habituation.

**DISCUSSION**

This ontogenetic study shows that maternal potentiation of isolation calling develops later than both the isolation calling response itself and the maternal quieting effect. Potentiation appears first at 7–9 postnatal days, in pups that have just interacted with an active dam for the longer of the two time periods tested (5 min). Contact with the passive dam does not potentiate until 4–6 days later, and it occurs first in the group with the shorter period of dam exposure (1 min). Peak levels of maternal potentiation are reached between 11 and 13 days of age, followed by a gradual decline of both isolation calling and maternal potentiation, until both responses disappear at 21–25 days of age. Other behavioral responses of pups to isolation such as locomotion and rising do not show maternal potentiation and have a very different developmental course, gradually increasing with age in their variety and frequency.

The pups' vocal responses during the brief periods of exposure to their dams follow a different developmental course from their potentiated responses following that exposure. In the 1st postnatal week, pups show a consistent inhibition of vocalization in response to both active and passive dams, the maternal quieting response. During the 2nd week pups become less consistent in this response, first with active dams and then even with passive dams, so that at age 13 days, none of the groups showed significant maternal quieting during their brief periods with her. Yet, these pups show just as intense potentiation of their subsequent isolation response as do younger pups that had responded to their dams with a marked reduction in their USV rates. Clearly, the potentiation effect cannot depend on the same processes that mediate the quieting effect of the dam’s olfactory and tactile cues. Finally, the behavior of active dams toward the pups during these brief interactions and the behaviors of pups toward their passive mothers do not show any ontogenetic changes that could account for the emergence of active or passive potentiation in the 2nd postnatal week. Although our previous work has shown that the passive potentiation response is stable over 1-hr periods after maternal removal from the home cage in 8-day-olds (Shair et al., 1997) and over 2 hr in 12-day-olds (Hofer et al., 1994), it remains possible that the fixed order of testing in these experiments (active dams first) may have influenced the comparisons between active and passive potentiation at other ages. Taken together, the results serve to dissociate the development of maternal potentiation from the development of the other, more familiar vocal responses of the pup (the isolation and contact quieting responses), from the development of the other behavioral responses of the pup to isolation, and from the development of the behavioral responses of dams and pups during their brief interactions. Thus, maternal potentiation appears to develop independently of these other responses and represents a new level of control over the pups’ vocalizations, one in which the pup’s response is related to temporal and qualitative features of previous experiences with the dam, not simply to the stimuli immediately present in the isolation environment.

In the 1st postnatal week, pups that are isolated after a period of maternal contact with active or passive dams show a continuation of the maternal quieting effect, a decrease in USV rates, instead of potentiation. This “carry-over” response is intuitively understandable as a prolonged effect of the previously induced state. But toward the end of the 1st week, brief maternal exposure appears to acquire a new “meaning” to the pup, now inducing an enhanced subsequent response to isolation. The pups have developed a new response to the same cues, a developmental event that may be the result of an experience-dependent process. Active maternal interaction produces the potentiation effect before passive contact, but this difference cannot be the result of the active dam simply stimulating the pups enough to induce a persistent arousal state. For even in its earliest appearance, active potentiation is a specific effect, limited to the pup’s vocal responses to isolation. Potentiation following passive maternal contact occurs 4–6 days later, suggesting that pups may require further experience in the litter situation before passive maternal cues, such as her odor, acquire the capacity to elicit potentiation. Indeed, we have recently found that olfactory denervation of 12-day-old pups eliminates passive but not active potentiation (Masmela, Shair, & Hofer, in press), revealing a difference in the sensory basis for the two forms of this response.

The pups’ initial USV responses to isolation show a developmental pattern consisting of increasing rates in the 1st week followed by decreasing rates thereafter, until no vocal response to isolation is present at the end of the 3rd postnatal week. This is the expected pattern and has been found in previous longitudinal studies (Graham & Letz, 1979; Noirot, 1968). USV rates in the 2nd postnatal week in the present study are somewhat lower than those reported in our previous
ous studies with this strain of rat, because in those we used arithmetic means that are elevated by the effect of a few pups with relatively high rates while the medians used in the present study are not. The decline in USV rates with age in the present study occurred coincidently with the rise of maternal potentiation and thus both trends could be part of a common developmental process, as will be discussed in the next two sections.

Another developmental trend coincides also with the rise of potentiation: the gradual waning of the maternal quieting response. It seems possible that pups gradually become capable of discriminating the passive dam, with her unavailable nipples, from their normal dam’s behavior. Pups may be responding to this discrepancy, as well as to the more familiar maternal cues, so that a mixed USV response to her presence ensues.

**An Adaptive Role for Maternal Potentiation?** The results of this present study are consistent with an evolutionary view in which an isolated pup’s calling rate is considered to have been selected to be responsive to cues in the pup’s recent past experience. As pups become more mobile in the 2nd week and the dam is away from the nest for longer periods, it becomes advantageous for pups to recognize cues that predict the relative likelihood of the dam being nearby and its calls eliciting a rapid maternal response, as distinct from cues that the dam may be at some distance and that its calls may also attract predators. Thus, recent behavioral interactions with an alert, responsive dam, as in active potentiation, or recent experience with her olfactory and tactile cues as in passive potentiation, predict a nearby dam and a rapid maternal response to a suddenly isolated pup. In this context, the highest possible calling rate is most likely to bring the dam back well before such calling attracts a predator. An evolved vocal response to predator cues in the infant rat is already known: the intense USV suppression and immobility that rat pups show in their first experience of their dam’s behavior. Pups may be responding to this discrepancy, as well as to the more familiar maternal cues, so that a mixed USV response to her presence ensues.

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Possible Learning Mechanisms. The earliest appearance of potentiation occurs after active maternal interaction, suggesting that we look for clues in the events of that second period in the potentiation sequence. We observed the dams licking, nosing and carrying their pups, repositioning them in the cage as well as hovering over them and moving away. These are precisely the behaviors that dams show when returning to the nest for each nursing bout. They have been studied in detail by Stern and Johnson (1989) and shown to be crucial for the elicitation of pup responses such as probing into the maternal ventrum that are, in turn, necessary for the dam to adopt the arched nursing position. These maternal behaviors not only activate dam-directed behaviors in pups, but they also can be viewed as salient and consistent predictive cues for the subsequent nursing period, and become powerful incentive cues as a result of repeated association with all
the subsequent reinforcing events of the nursing bout. An expectancy may thus be created that is then violated by the abrupt isolation of the pup in the final period of the potentiation sequence. In other words, a cue predicting high levels of reinforcement is followed instead by a (presumed) low-reinforcement experience, isolation, a sequence sometimes referred to as “negative contrast.” These are conditions that, in other settings, have been shown to generate high levels of adrenocortical output (Bayart et al., 1990) and behaviors termed “primary frustration” (as reviewed by Amsel, 1992). This response could summate with the isolation response, producing a potentiated USV response.

Support for this learning hypothesis, as applied to rat pup USV, comes from a study on extinction of an appetitive learning task in 12-day-old rats (Amsel, Radek, Graham, & Letz, 1977). Pups were trained to move rapidly down a runway to receive, on each trial, the opportunity to suckle an anesthetized dam in a goal box for 30 s. Pups’ USV rates while alone in the novel apparatus gradually fell to low levels as they learned to move directly and rapidly down the runway to the dam. The dam was then removed from the goal box. On the next trial, pups ran to the end of the runway, but finding no dam, began vocalizing at high rates while remaining near the empty goal box. The high USV rates were interpreted by the authors as evidence of “primary frustration.” This study shows that an expectancy can be learned by 12-day-old rat pups on the basis of repeated maternal exposure and that violation of that expectancy does in fact increase USV rates in isolated rat pups of an age at which we find the potentiation response to be near its peak.

Another finding of the present study is also consistent with this learning hypothesis: the gradual decrease in the initial response to isolation during the 2nd postnatal week. The context of littermates without the dam present should gradually be learned to predict low levels of the immediate expectancy of the reinforcing events of the nursing bout. A pup that becomes isolated from this context (as in the initial isolation periods of the present study) would experience relatively little violation of expectancy and emit relatively low USV rates.

We cannot, however, assume that an expectancy is learned primarily in relation to the reinforcements of nipple attachment, suckling, and milk letdown. Pups suckling on an anesthetized dam show no more potentiation when subsequently isolated than those merely in contact with her flank (Hofer et al., 1996). And the decline in potentiation following 30–60 min suckling an anesthetized dam is not prevented or even reduced by the repeated provision of milk to the pups through oxytocin-induced maternal milk ejections. Finally, the acquisition of potentiation by males through biparental rearing (Brunelli et al., in press) raises questions about any hypothesis based on associations formed during nursing itself. Other parent–infant interactions, such as stepping, vigorous licking, and stroking, however, can be reinforcing in themselves, particularly during the 1st postnatal week (Sullivan, Hofer, & Brake, 1986). Thus, it seems possible that cues are learned and expectancies generated in relation to caretaking responses rather than nursing. Males reared with pups participate in these caretaking activities and might thus acquire the capacity to potentiate pups’ USV responses in this way. But despite the appeal of these learning hypotheses, it must be kept in mind that natural selection may well have facilitated the development of potentiation through other (unlearned) processes.

In conclusion, it may be possible to propose a naturally occurring human analogy to maternal potentiation, as suggested by one of us (J. R. M). If the mother of a toddler returns briefly to the day-care center for some reason soon after dropping off her child on the way to work and then starts to leave again after this brief visit, she may be startled by a frantic outburst of crying from her child, very unlike the mild response to her initial departure earlier in the day. This intense vocal response to the second separation can be inexplicable to the mother and is a powerful inducement to give up her plans for the morning and remain with her child.

NOTES

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REFERENCES


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