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Anticipation of Play Elicits High-Frequency Ultrasonic Vocalizations in Young Rats

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The authors provide initial documentation that juvenile rats emit short, high-frequency ultrasonic vocalizations (high USVs, ~55 kHz) during rough-and-tumble play. In an observational study, they further observe that these vocalizations both correlate with and predict appetitive components of the play behavioral repertoire. Additional experiments characterized eliciting conditions for high USVs. Without prior play exposure, rats separated by a screen vocalized less than playing rats, but after only 1 play session, separated rats vocalized more than playing rats. This finding suggested that high USVs were linked to a motivational state rather than specific play behaviors or general activity. Furthermore, individual rats vocalized more in a chamber associated with play than in a habituated control chamber. Finally, congruent and incongruent motivational manipulations modulated vocalization expression. Although play deprivation enhanced high USVs, an arousing but aversive stimulus (bright light) reduced them. Taken together, these findings suggest that high USVs may index an appetitive motivation to play in juvenile rats.

The analysis of spontaneous vocalizations has provided a robust method for making inferences about the emotional experience of mammals (e.g., see Newman, 1988; Panksepp, Siviy, & Normansell, 1985). Although most of the work has focused on vocalizations that are within the human auditory range (20 Hz–20 kHz), more recent investigations have focused on decoding the very high frequency vocalizations (>20 kHz) exhibited by many rodents. Such ultrasonic vocalizations (USVs) have been documented in the context of several different types of rodent social encounters, including aggressive, sexual, and parental interactions (Sales & Pye, 1974). In the case of rats, USVs emitted during agonistic social encounters have received extensive ethological description (Miczek, Tornatzky, & Vivian, 1991). For example, during resident-intruder aggression between adult males, intruders emit both *low* (20–25 kHz, monotone) and *high* (40–70 kHz, frequency modulated) USVs.

Intruders primarily make low USVs during submissive behaviors (i.e., defensive upright or submissive supine postures; Corrigan & Flannelly, 1979) and generally emit high USVs during approaching or aggressive behaviors but rarely after defeat (Thomas, Takahashi, & Barfield, 1983). Furthermore, the presence of predators can potently evoke low (but not high) USVs in hiding adults when conspecifics are nearby (Blanchard, Blanchard, Agullana, & Weiss, 1991). Finally, infant rats also emit characteristic *midrange* (40 kHz) vocalizations when separated from their caregiver. Blumberg and Alberts (1991) have hypothesized that these midrange infantile vocalizations are analogous to low adult USVs, and they postulate that the difference in frequency results from the relative smallness of the infantile larynx. In sum, various ethological observations suggest that aversive social events consistently evoke low USVs in adult rats.

Appetitive social encounters also have been reported to elicit USVs in rats. For example, prior to copulation, adult rats emit short, high USVs (McIntosh & Barfield, 1980). Male rats' production of these vocalizations functionally enhances the solicitation behaviors of estrous females (e.g., darting and ear-wiggling; Thomas, Howard, & Barfield, 1982). Thus, unlike low USVs, high USVs appear to mark and facilitate at least one type of appetitive social behavior in adult rats.

A number of functional explanations can be offered to account for the incidence of high USVs in the context of rat social interaction. These accounts provide explanations at one of roughly three functional levels: (a) physiological—high USVs are an artifact of motor activity (i.e., are produced by the thoracic compression that accompanies forepaw impact; Blumberg, 1992); (b) motivational—high USVs are the by-product of a motivational or emotional state (i.e., Bell, 1974; Vivian & Miczek, 1991); or (c) social—high USVs enable individual rats to communicate and thereby to coordinate social interactions (Barfield & Thomas, 1986). Certainly, these different levels of explanation are not mutually exclusive. For instance, high USVs appear during active segments of social interactions, supporting an activity account (Blumberg, 1992). They also occur in the context of appetitive behavior, supporting a motivational account (McIntosh & Barfield, 1980). Finally, they have

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been shown to facilitate copulation, supporting a social account (Thomas et al., 1982). Nonetheless, different behavioral predictions can be generated for each level of functional analysis. For instance, from a motivational perspective, if high USVs mark an appetitive motivational state, then they also should appear in the context of other appetitive social behaviors besides copulation.

Rough-and-tumble play represents an unconditioned and vigorous form of social interaction shown by juvenile rats of both sexes that diminishes after the onset of puberty (Panksepp, 1981). Previously isolated juvenile rats show vigorous play behavior when placed together in a chamber for brief periods of observation. Rats first direct play attacks toward the nape of each others' necks, often accompanied by placing forepaws on the back of one's opponent (*dorsal contacts*). An attacked rat will rotate to a supine position to defend its neck against these dorsal contacts, at which point the attacker can "pin" the defender to the ground momentarily (see Pellis & McKenna, 1992; Siviy & Panksepp, 1987). Until now, USVs have not been analyzed in the context of the play of juvenile rats or of other rodent species. Because the opportunity to play is a positive incentive for juvenile rats (Calcagnetti & Schechter, 1992; Normansell & Panksepp, 1990), if rats do vocalize in the context of play, one might predict that they predominantly emit high rather than low USVs.

Experiment 1

Experiment 1 was conducted to establish whether play would elicit more high USVs than solitude.

Method

Subjects. Subjects were 26 hooded Long Evans rats 22 days old (12 males, 14 females), born in the vivarium of the Department of Psychology, Bowling Green State University (Bowling Green, OH). All subjects had been individually housed after weaning at 20 days to maximize play motivation (Panksepp, Siviy, & Normansell, 1984). Subjects had ad libitum access to food and water, were housed on a 12:12 light–dark schedule, and participated in the experiment during the latter half of their light phase.

Procedure. Testing took place in two identical 31 × 32 × 32 cm lucite test cages (the "play" chamber and "control" chamber), which were situated in adjacent soundproof chambers and illuminated with 25 W red lights. During testing, two Mini-3 Bat Detectors (Ultra Sound Advice, London) were installed in the ceiling of the relevant chamber and tuned to 30 kHz (sensitivity range = 20–40 kHz) and 55 kHz (sensitivity range = 45–65 kHz), respectively. Outside the chamber, coders listened to high and low USVs by using remote headphones and recorded them on a manual counter. By listening to the low-frequency Bat Detector in the left earphone and the high-frequency Bat Detector in the right earphone, a single coder could simultaneously code low and high vocalizations. To ensure that the USVs could be reliably measured in this manner, two independent coders scored the initial vocalizations of each subject for 30 s in the play chamber. High intercoder agreement was obtained (Spearman $\rho = .90$, $Z = 5.17$, $p < .001$).

For each of 3 consecutive days, pairs of subjects were placed in the play chamber and allowed to play together for 120 s while their vocalizations were recorded. Pair composition was determined by a round-robin matching scheme without replacement so that subjects always played with a novel partner, in order to control for potential influences of social familiarity. Each day, subjects also were placed alone in the control chamber for 120 s while their vocalizations were recorded. The litter was not changed in either of the chambers over the course of testing in order to maintain control of potential olfactory cues. Order of testing (e.g., play chamber, then control chamber, or vice versa) was counterbalanced over days, and tests occurred successively in both chambers, following either a 0- or 120-s delay.

Results

USVs were analyzed with 2 (chamber, within pair) × 2 (order) repeated measures analyses of variance (ANOVAs). All analyses were conducted on paired data. Specifically, a pair's vocalizations during play were compared with the sum of both partners' individual vocalizations while alone for the same amount of time in the control chamber. Because pair composition varied across days, separate analyses were performed on each day's data.

As summarized in Figure 1, on Day 1 there were no effects of chamber on high USVs. This was not surprising, because previously isolated rats typically require a day of habituation to an environment before exhibiting full play activity. However, there was a significant effect of chamber on low USVs, indicating that rat pairs emitted more low USVs while alone in the control chamber ($M \pm SEM = 81.50 \pm 22.51$) than while playing together (29.00 ± 14.13), $F(1, 10) = 7.03$, $p < .05$. From these data alone, it is not clear whether isolation in a novel environment increased low USVs in the control chamber or, conversely, whether the presence of a partner decreased low USVs in the play chamber.

On Day 2, the predicted pattern of findings emerged. A main effect of chamber on high USVs indicated that subjects made more high USVs in the play chamber (176.33 ± 24.55) than in the control chamber (88.92 ± 13.91), $F(1, 10) = 7.90$, $p < .05$. However, there were no significant effects of chamber on low USVs. On day 3, a similar main effect for chamber on high USVs indicated that subjects again emitted more high USVs in the play chamber (181.83 ± 25.37) than in the control chamber (118.67 ± 12.37), $F(1, 10) = 5.41$, $p < .05$, and there was no effect of chamber on low USVs. No significant main effects or interactions of test order emerged in any of the analyses.

Discussion

After an initial day of habituation, pairwise analyses indicated that rats made more high USVs while playing together than while alone in a control chamber for an equivalent length of time. Low USVs did not differ for play versus isolation treatments. These findings suggest that play can specifically elicit high USVs. However, they do not clarify the functional role of the vocalizations during play activity. If the vocalizations mark an appetitive aspect of play, then they should be correlated with and perhaps even predict other specific appetitive behaviors in the play behavioral repertoire. As mentioned earlier, play consists of both dorsal contacts and pins, and dorsal contacts (but not

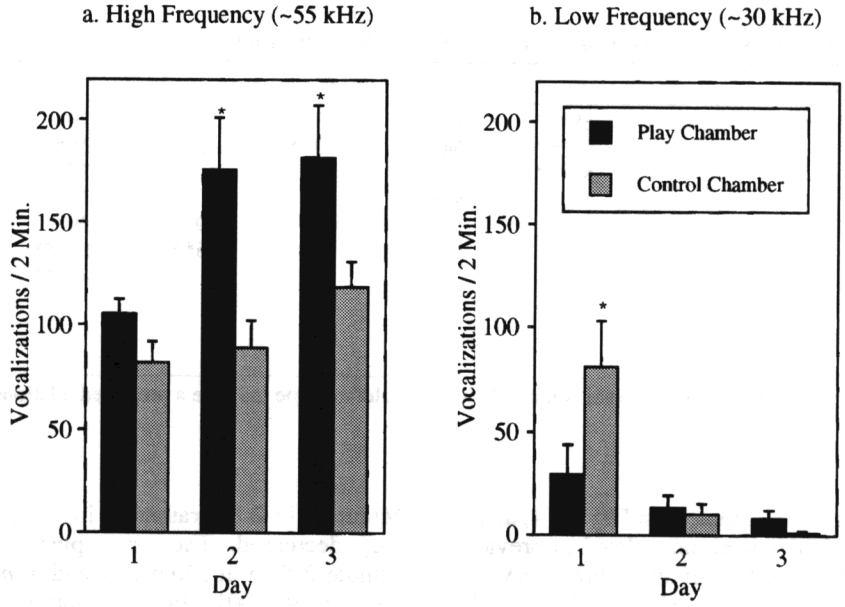

Figure 1. Ultrasonic vocalizations over 3 days of play for rat pairs ($M \pm SEM$). *$p < .05$.

pins) have been interpreted as an index of play motivation (Panksepp et al., 1984). Thus, we conducted a second observational study to test whether high USVs would correlate with dorsal contacts within play sessions and might predict dorsal contacts on subsequent days.

Experiment 2

In Experiment 2, we examined whether high USVs would correlate with appetitive play behaviors and whether high USVs on a given day would predict appetitive play behaviors on subsequent days. Additionally, this study provided a minute-by-minute summary of the incidence of high USVs in relation to other play behaviors during a typical play session.

Method

Subjects. Subjects were 52 hooded Long Evans rats 21 days old (26 males, 26 females), raised in the Bowling Green vivarium and cared for as described in Experiment 1.

Procedure. Observation occurred in a 31 × 32 × 32 cm lucite test cage that was situated in a soundproof chamber illuminated with a 25 W red light, as in Experiment 1. Pairwise play behaviors and high USVs (45–65 kHz) were recorded to videotape by using a Bat Detector installed in the ceiling of the chamber for later coding and analysis.

All of the rats were individually placed in the play box for 5 min the day before testing in order to habituate them to the testing arena. On each of 5 subsequent test days, subject pairs were placed in the chamber for 300 s and were videotaped. Unlike in Experiment 1, pair composition remained constant over the five play sessions to enable correlation of pairwise behavioral counts across days.

Videotaped play sessions were coded for incidence of dorsal contacts (one partner places its paws on the other's dorsum), pins (one partner lies with its dorsal surface to the ground, typically with the other's paws on its ventrum), and high USVs. Inspection of the videotapes revealed that subjects expressed two types of high USVs: mainly short (<1 s) and, much less frequently, long (>1 s; observed in only 4 of 26 pairs). These types of vocalizations were scored separately, as has been the practice of other investigators (Blanchard, Yudko, Blanchard, & Taukulis, 1993).

We hypothesized that if short, high USVs marked an appetitive motivation to play, they would correlate with dorsal contacts, but not with pins, during each play session. Additionally, some rat pairs consistently emitted more play behaviors than others after the first few days of play. Thus, we hypothesized that if short, high USVs provide an earlier marker of play motivation than dorsal contacts within a specific pair, then vocalizations on a given day should predict dorsal contacts on later days (but that dorsal contacts should not conversely predict later short, high USVs).

Results

Dorsal contacts; pins; short, high USVs; and long, high USVs were summed for each 300-s play session, and these sums were then correlated. As predicted, correlations revealed that short, high USVs were unrelated to pins at all time points but were significantly related to dorsal contacts (see Table 1). Specifically, short, high USVs were significantly related to dorsal contacts on Days 2, $r(25) = .65, p < .01$, and 4, $r(25) = .45, p < .05$. The relationship was not reliable on Day 3, and there was only a nonsignificant trend for short, high USVs to be related to dorsal contacts on Day 5, $r(25) = .37, p < .10$. The lack of a correlation on Day 1 is not surprising given that juvenile rats typically require a day to habituate to and fully express play activity (as exemplified by the lack of differences in the vocalizations of playing versus isolated rats on Day 1 of Experiment 1).

More important, short, high USVs on Days 2–4 significantly predicted dorsal contacts on all subsequent days, $rs(25) = .39–.51$, $ps < .05$. For instance, short, high USVs

Table 1
Intercorrelations of High USVs With Dorsal Contacts Over 5 Days for Rat Pairs

Variable	1	2	3	4	5	6	7	8	9	10
1. USV1		.51***	.60***	.58***	.71***	.23	.17	−.10	.14	.29
2. USV2			.72***	.57***	.64***	.40**	**.65*** **	**.39** **	**.41** **	**.51*** **
3. USV3				.84***	.85***	.13	.41**	**.25**	**.50*** **	**.47** **
4. USV4					.84***	.12	.30	.19	**.45** **	**.39** **
5. USV5						.03	.29	.09	.30	**.37***
6. DC1							.65***	.55***	.59***	.59***
7. DC2								.84***	.65***	.73***
8. DC3									.69***	.61***
9. DC4										.78***
10. DC5										

Note. USV = ultrasonic vocalization; DC = dorsal contact. Values in boldface type indicate a predicted relationship.
*$p < .10$. **$p < .05$. ***$p < .01$.

on Day 2 predicted later dorsal contacts on Days 3, 4, and 5. Conversely, short, high USVs were not related to previous dorsal contacts, with the exception of short, high USVs on Day 2 with dorsal contacts on Day 1, $r(25) = .40, p < .05$, and short, high USVs on Day 3 with dorsal contacts on Day 2, $r(25) = .41, p < .05$. Thus, with a few exceptions, the correlational evidence appears to favor the prediction that high USVs during one play session predict dorsal contacts during the next.

Because only 4 of 26 pairs of rats emitted long (>1 s), high USVs over the 5 days of testing, the behaviors of these pairs were compared with those of pairs that made no long, high USVs, by using *t* tests. Although pairs that emitted long, high USVs did not make any fewer pins than pairs that did not, they did make fewer dorsal contacts on Day 2, $t(24) = 3.44, p < .005$; Day 3, $t(24) = 3.29, p < .005$; Day 4, $t(24) = 3.09, p < .005$; and Day 5, $t(24) = 2.48, p < .05$. So, unlike short, high USVs, the presence of long, high USVs appeared to mark pairs that consistently made fewer dorsal contacts overall. These findings provide additional functional justification for the practice of separately scoring long versus short, high USVs.

Minute-by-minute plots of the play behaviors over each play session indicated a similar behavioral profile for each daily play session. Therefore, Day 3 was taken as a representative play session and subjected to a 2 (sex) × 5 (minute-within) analysis of variance (ANOVA) with dorsal contacts, pins, and high USVs as outcome variables. Post hoc comparisons were performed with Tukey's least significant difference (LSD; within-subjects comparisons were based on the error terms for the overall ANOVA effects). These analyses revealed a significant diminution of all three play behaviors over the course of the session, as seen in Figure 2. Post hoc comparisons indicated that high USVs dropped significantly from Minute 1 to Minute 2 ($p < .001$), whereas high USVs during subsequent minutes did not significantly differ, $F(4, 100) = 14.29, p < .001$. Similarly, post hoc comparisons revealed that dorsal contacts dropped significantly from Minute 1 to 2 ($p < .001$), but also from Minutes 2 and 3 to Minute 5, $ps < .05$; $F(4, 100) = 12.14$, $p < .001$. Although pins also diminished over the play session, they did not show a significant decrease from Minute 1 to 2 but rather an increase ($p < .05$), after which they decreased. Thus, rats pinned significantly more at Minute 2 than at Minutes 4 and 5, $p < .001$; $F(4, 100) = 5.93$, $p < .001$. In sum, although all play behaviors decreased over the course of the session, both high USVs and dorsal contacts showed large drops following Minute 1, whereas pins steadily decreased later in the session.

Discussion

Analyses consistently supported the hypothesized relationship between short, high USVs and appetitive play behaviors. Following Day 1, short, high USVs were correlated with dorsal contacts but not pins. The summary nature of our analyses do not clarify the exact temporal relationship of vocalizations to dorsal contacts, but this issue may be addressed in the future with slow-motion microanalytic analysis of these behaviors (e.g., see Pellis & Pellis, 1991). Regardless, short USVs appeared to covary with dorsal contacts.

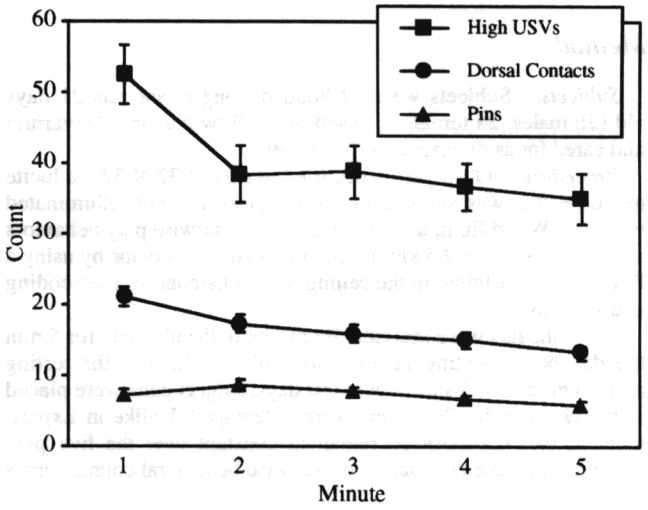

Figure 2. High ultrasonic vocalizations (USVs), dorsal contacts, and pins over 5 min for rat pairs ($M \pm SEM$).

Furthermore, short, high USVs at a given time point predicted dorsal contacts on later days but were not related to dorsal contacts on previous days (with two exceptions). This finding implies both that high USVs may provide a more sensitive measure of pairwise play motivation than dorsal contacts and that playing rats retain and act on memories of prior interactions with the same partner. The fact that short, high USVs predict dorsal contacts on later days also suggests that they are not merely a by-product of other appetitive play behaviors.

Finally, repeated measures analyses indicated that although all play behaviors diminished over the course of a representative play session, both high USVs and dorsal contacts showed a clear drop after Minute 1, whereas pins decreased later in the play session. These descriptive findings reinforce the observed correlation between high USVs and dorsal contacts. Although this experiment suggests that high USVs are not an artifact of appetitive play behavior, they still may stem from high levels of general activity that accompany play. Thus, Experiment 3 was conducted to determine whether play behavior or general activity was necessary to elicit high USVs.

Experiment 3

Experiment 3 was designed to determine whether rats would emit more high USVs during play than during conspecific exposure without play activity. In addition to measuring high vocalizations during a play session, we also measured gross motor activity. We predicted that if high USVs marked an anticipatory state rather than a motor artifact, they should continue at a high rate while rats were exposed to each other without an opportunity to play.

Method

Subjects. Subjects were 64 hooded Long Evans rats (32 males, 32 females) raised in the Bowling Green vivarium and cared for as described in Experiment 1. Subjects had been independently housed at 21 days of age to enhance play motivation and were 31 days old at the time of testing. All animals were individually placed in the play box for 5 min the day before testing in order to habituate them to the testing arena.

Procedure. Testing occurred in a 31 × 32 × 32 cm lucite test cage that was situated in a soundproof chamber illuminated with a 25 W red light, as in Experiment 1. During testing, the chamber took on one of two configurations. In the play configuration, both subjects were placed in the chamber together as described earlier. In the screen configuration, subjects were placed on either side of a 0.5-cm gridded hardware cloth screen that separated the pair by bisecting the chamber, and high USVs (45–65 kHz) were recorded as before. Total rough-and-tumble play activity per pair was also recorded at a gain of 2 with an inductance-based activity platform (Stoelting, Inc, Kiel, WI) that served as the floor of the play chamber.

For 2 consecutive days, subject pairs were placed in the chamber for 300 s as their vocalizations and activity were recorded. Pair composition remained constant over both days of testing. Each pair was randomly assigned to one of four different conditions. In the two experimental conditions, subjects either played the first day and were separated by the screen the second day (play/screen) or they were separated by the screen the first day and played the second day (screen/play). In the two control conditions, subjects either played both days (play control) or were separated by the screen both days (screen control).

This design allowed us to contrast the activity and the motivational accounts of high USVs. In line with the motivational account, we made two predictions: (a) Screened rats would vocalize at an equally high rate as playing rats only if they anticipated play (i.e., had played the day before); and (b) although they might vocalize as much as playing rats, screened rats would show less general activity than playing rats. Thus, for rats who were separated on Day 2 after playing on Day 1, we predicted a dissociation of high USVs and motor activity.

Results

Both high USVs and activity during the 300-s play sessions were analyzed with 4 (condition) × 2 (day, within pair) repeated measures ANOVAs. Post hoc comparisons were performed with Tukey's LSD. All analyses were conducted on paired data. Preliminary analyses with sex as a factor yielded no main effects or interactions related to sex, so that factor was not included in the analyses that follow.

As seen in Figure 3, a main effect for condition indicated that subjects in different conditions varied in their overall production of high USVs, $F(3, 28) = 8.23, p < .001$, and a main effect of day, $F(1, 28) = 20.38, p < .001$, indicated that high USVs increased overall from Day 1 (387.81 ± 63.11) to Day 2 (507.69 ± 68.70), but both of these main effects were qualified by the predicted interaction of condition and day, $F(3, 28) = 4.32, p < .05$. Post hoc analyses indicated that the two experimental groups (separate/play and play/separate) both made significantly more vocalizations on Day 2 ($ps < .001$). On Day 1, the playing conditions vocalized more than the separated conditions ($p < .05$). However, on Day 2, although the separate control continued to vocalize less than the play control ($p < .001$), the number of vocalizations in the separate/play condition rose to the same number as in the play control condition ($p < .001$), whereas the play/separate condition contained significantly more vocalizations than both of these playing groups in spite of the subjects' separation and resultant inability to play ($p < .001$). Increased vocalizations in both experimental groups largely accounted for the overall increase in vocalizations on Day 2.

Analysis of activity scores also supported the predictions. As seen in Figure 3, a main effect of condition, $F(3, 28) = 16.37, p < .001$, indicated that subjects in different conditions varied in general activity, whereas a main effect of day, $F(1, 28) = 4.60, p < .05$, revealed an increase in activity from Day 1 (238.34 ± 78.64) to Day 2 (307.41 ± 88.80). But again, the predicted interaction of condition and day qualified these main effects, $F(3, 28) = 21.55, p < .001$. Although post hoc analyses revealed that only the experimental conditions changed significantly from Day 1 to Day 2, those conditions changed in opposite directions, with the separate/play condition increasing in activity and the play/separate condition decreasing in activity. On both Days 1 and 2, playing groups were more active than separated groups ($p < .001$). Taken together, these analyses reveal a dissociation of vocalizations and activity on Day 2 for the

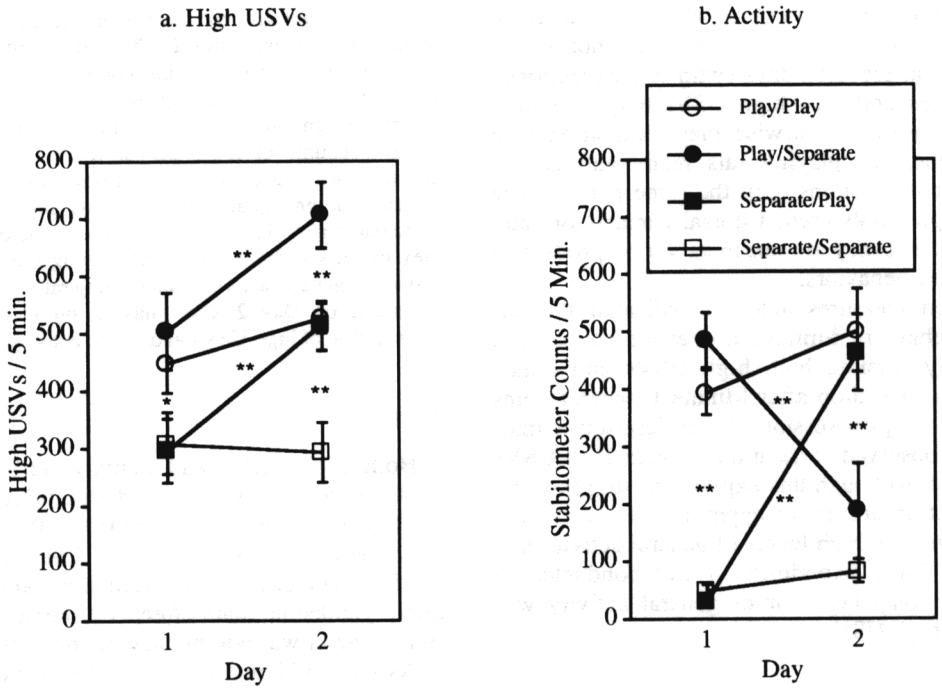

Figure 3. High ultrasonic vocalizations (USVs) and activity for play versus conspecific exposure ($M \pm SEM$). $^{*}p < .05$. $^{**}p < .01$.

play/separate group only; vocalizations increased in spite of reduced activity.

Discussion

Even during decreased activity, separated rat pairs that had played earlier vocalized more than playing controls. Conversely, separated rat pairs that had not played before vocalized less than playing controls. This contrast suggests both that only one trial of play is required to induce a motivational state that evokes high USVs in rats and that general motor activity alone cannot account for the expression of these vocalizations (e.g., Blumberg, 1992). This finding further suggests that anticipation of play may elicit high USVs even more powerfully than play activities themselves. However, if these vocalizations mark a motivational state relevant to play anticipation, the presence of a conspecific should not be required for their expression; cues associated with play also should elicit the vocalizations in individuals. Thus, in Experiment 4, we examined whether a chamber associated with play could conditionally elicit high-frequency USVs in individual subjects.

Experiment 4

Experiment 4 was designed to test whether individual rats would emit high-frequency USVs in anticipation of play but in the absence of any play partners.

Method

Subjects. Subjects were 20 hooded Long Evans rats (14 males, 6 females) 28 days old, born and cared for as described earlier. All subjects had been weaned and individually housed at 22 days of age.

Procedure. Testing took place in both the play and the control chambers used in Experiment 1. For 3 consecutive days prior to the test day, subjects were exposed to both the play and the control chambers in counterbalanced order. Each day, subjects were placed alone in the control chamber for 300 s. Similarly, subjects played with a novel partner for 300 s in the play chamber each day. As in Experiment 1, pair composition was determined by a round-robin selection scheme without replacement. On the fourth day, individual subjects' high-frequency USVs were recorded for 30 s successively in both the play and the control chambers in counterbalanced order. The interval between the two tests was 30 s.

Results

High USVs emitted during the 30-s recording sessions were analyzed with a 2 (chamber, within subject) × 2 (order) × 2 (sex) repeated measures ANOVA. As seen in Figure 4, a main effect for chamber indicated that all subjects vocalized more in the play chamber (36.30 ± 2.75) than in the control chamber (12.90 ± 2.66), $F(1, 16) = 124.63$, $p < .001$. An interaction for Sex × Chamber indicated that this effect was more pronounced for female rats, $F(1, 16) = 5.84$, $p < .05$, but this could be attributed largely to females' reduced levels of vocalizations in the control chamber. As in Experiment 1, there were no significant main effects or interactions of test order.

Discussion

Even in the absence of a partner, all subjects made more high-frequency USVs in a chamber where they had previ-

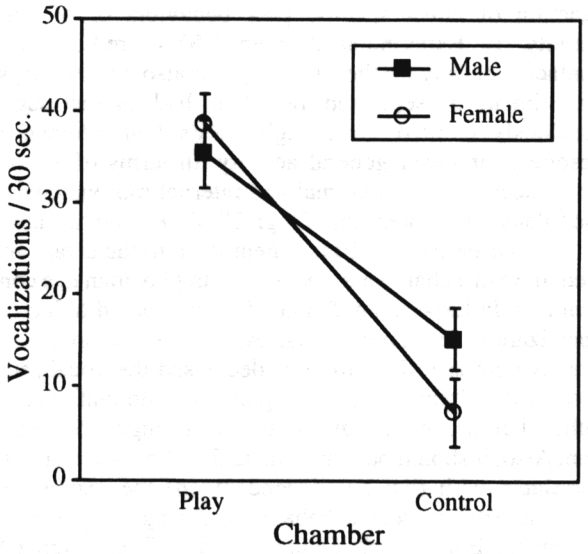

Figure 4. High ultrasonic vocalizations in play versus control chambers for individual rats ($M \pm SEM$).

ously played than in a control chamber. Because the chambers were similar in terms of visual, auditory, and tactile cues, it is likely that the subjects distinguished them by olfactory cues yet to be identified. Association of high USVs with the play environment rather than the partner per se supports the hypothesis that these vocalizations are linked to a motivational state engendered by the anticipation of play. To further evaluate this hypothesis, we manipulated play motivation by comparing the high USVs of subjects housed individually versus in a group during a bout of play.

Experiment 5

Experiment 5 was designed to evaluate whether increased play motivation enhances the expression of high-frequency USVs. Thus, we compared the high-frequency USVs of subjects housed in a group versus individually during 2-min encounters with unfamiliar peers. Because social deprivation enhances play activity, presumably by increasing an appetitive motivation to play (Panksepp et al., 1984), we reasoned that individually-housed subjects would show more high USVs and play behaviors than group-housed subjects during an initial encounter with a conspecific.

Method

Subjects. Subjects were 16 hooded Long Evans rats (8 males, 8 females) 33 days old, born and cared for as described earlier. Half had been weaned and individually housed at 18 days of age ("isolate-housed"); the other half had lived in the home cage with their mother and littermates until testing ("socially housed"). All subjects had experienced 10 daily 300-s sessions of play 1 week prior to participating in Experiment 5.

Procedure. Subjects were paired with an unfamiliar partner of the same condition and sex (e.g., isolate male with isolate male). Pairs were placed in the play chamber described in Experiment 1 for 120 s, and high-frequency USVs were recorded.

Results

Scrutiny of the data revealed unequal variances between the socially housed and isolate-housed groups for high USVs, because one socially housed pair made far more vocalizations during play than any of the other socially housed pairs. Thus, a separate variance t test was used in the analysis of group differences in vocalization. This test indicated that isolate-housed pairs emitted significantly more high USVs (182 ± 3.21) than did socially housed pairs (71 ± 27.54), $t(3) = 4.00, p < .05$. Similarly, isolate-housed pairs made more dorsal contacts (40.8 ± 1.42) than did socially housed pairs (14.8 ± 1.42), $t(6) = 7.74, p < .05$. No isolate pairs and only one social pair made any pins.

Discussion

These findings indicated that previously socially isolated animals emit more high-frequency USVs than do group-housed animals during play. Presumably, this difference in vocalizations results from increased play motivation on the part of the individually housed animals. This interpretation concurs with an observed increase in dorsal contacts of the individually housed animals.

High USVs might appear as a result of any of several distinct motivational states relevant to the anticipation of play. For instance, one might hypothesize that the prospect of play evokes a high state of general arousal in juvenile rats, which in turn elicits vocalizations (e.g., Bell, 1974). However, a more specific hypothesis might posit that the vocalizations index a valenced state, such as an appetitive motivation to play. Because stimuli such as bright lights are thought to induce an aversive state of autonomic arousal in juvenile rats (e.g., Kurtz & Campbell, 1994) and also have been shown to reduce play activity (Panksepp et al., 1984), a general arousal hypothesis would predict that such a stimulus either should not affect conditioned vocalizations or should increase them. On the other hand, a more specific appetitive motivation hypothesis would predict that bright light should reduce conditioned vocalizations.

Experiment 6

To distinguish between general-arousal and appetitive-motivation accounts of high-frequency USVs, we compared anticipatory vocalizations in the play chamber in the presence of a bright white light versus the dim red light used in prior experiments.

Method

Subjects from Experiment 4 participated in Experiment 6 after 4 more days of dyadic play in the play chamber and individual exposure to the control chamber. Experiment 6 was conducted in the play chamber only. In the high-illumination condition, a bright 200 W lightbulb was placed in the play chamber 5 cm away from the front lucite wall (yielding 1,500 Lux in the chamber), and high-frequency USVs were recorded for 30 s. In the control condition, the bright light was turned off, leaving only the same dim red illumination that was used in Experiments 1–5 (25 W, <5

Lux). High illumination and control conditions were run on consecutive days in counterbalanced order across subjects to reduce any potential within-day effects of fear priming.

Results

A 2 (lighting, within subject) × 2 (order) × 2 (sex) repeated measures ANOVA revealed a main effect for lighting, $F(1, 16) = 21.24, p < .001$, indicating that subjects vocalized less in the high-illumination condition (17.50 ± 3.29) than in the control condition (33.75 ± 3.28). There were no significant main effects or interactions of sex or order.

Discussion

Bright light reduced conditioned vocalizations to control levels observed in Experiment 4, supporting an appetitive-motivational but not a general-arousal hypothesis. Additionally, these data are consistent with the possibility that elevated aversive motivation induced by the bright light may reduce high USVs.

General Discussion

In summary, Experiment 1 provided documentation that play elicits more frequent high USVs in juvenile rats when compared with isolation testing. Experiment 2 indicated that the vocalizations selectively covaried with and predicted appetitive play behaviors, as indexed by dorsal contacts. Experiment 3 suggested that rats vocalized even more when precluded from play behavior, but only if tested under conditions in which the expectation of play was presumably high. Experiment 4 demonstrated that rats vocalized more in a place where they had played previously than in a place where they had not, which suggested that the presence of a play partner was not necessary to elicit the vocalizations. Together, these four experiments implied that high USVs marked a motivational state associated with play (rather than behavioral activation) in juvenile rats.

Experiments 5 and 6 further elucidated some motivational aspects of high USVs. In Experiment 5, rats with enhanced play motivation due to individual housing vocalized more than group-housed rats. In Experiment 6, an arousing but mildly aversive stimulus (bright light) reduced the expression of high USVs in the play chamber relative to a presumably nonaversive stimulus (dim light). This finding suggested that high USVs do not simply arise from general arousal but rather from a more specific appetitive motivational state related to play or anticipation of other forms of appetitively motivated social interaction. This result also concurs with our nonexperimental observations that rats emit the high USVs while interacting with a familiar experimenter but remain silent in the presence of an unfamiliar experimenter (i.e., both experimenters wiggled their fingers in front of subjects in their home cages and occasionally brushed their noses).

These data clarify some issues regarding the functional role of high USVs in the play of juvenile rats. In terms of an activity account, high USVs do not occur solely as a function of either specific play behaviors or of general activity. In Experiment 2, high USVs predicted dorsal contacts not only on the same day but also on later days. In Experiment 3, separated rats that had played together previously emitted more high USVs than playing rats, despite their lower general activity. In terms of a motivational account, both external and internal motivational cues modulated the expression of high USVs, even in the absence of a social partner. In Experiment 4, individual rats vocalized more in a chamber associated with play than in a control chamber. In Experiment 5, play deprivation led to increased vocalization in playing rats. And in Experiment 6, an aversive but arousing stimulus decreased the vocalizations, specifically implicating an appetitive motivational state rather than a general state of arousal in high-USV production. Also, it should be emphasized that the vocalizations are correlated with (but not dependent on) the expression of other appetitive social behaviors during play (see also McIntosh, Barfield, & Thomas, 1984). Thus, high USVs may lead to reciprocation and subsequent intensification of play behaviors. However, more characterization of the social function of these vocalizations is needed, particularly in terms of determining whether recorded vocalizations can facilitate specific play behaviors. Overall, our findings support both motivational and social accounts of the function of high USVs during social play. Future experiments assessing the presence of these high USVs in nonsocial but rewarding scenarios should help further disentangle motivational from social accounts.

In agreement with the present results, where we observed high levels of USVs under conditions where subjects could not physically interact (i.e., Experiments 3 and 5), previous investigators have observed similar phenomena. Peters, Koch, Blythe, and Sufka (1988) observed that male rats exhibited elevations of long, low (22 kHz) calls when confronted by a female toward whom copulatory behavior had been inhibited in the male by previous associations of their social overtures with LiCl-induced illness. However, high USVs were not inhibited in these same animals. Also, it is noteworthy that high USVs have been observed in rats (particularly females) when confronted by anesthetized conspecifics, suggesting that this high-frequency call may reflect a desire for social engagement even if active social behavior is not possible (Blanchard et al., 1993). The 55 kHz vocalizations observed in the present studies are probably the juvenile counterpart of the high-frequency vocalizations observed in the studies just mentioned.

The high USVs observed in the present studies show both differences and similarities with other USVs documented in the social encounters of younger and older rats. For instance, the high frequency and short duration of these vocalizations clearly differentiate them from distress vocalizations seen in preweanling rats who have been separated from their mother and littermates (Noirot, 1972). Similarly, the frequency and length of these vocalizations also differentiate them from those observed in adult rats that have been defeated in a fight (Sewell, 1967). However, the high USVs observed in play do resemble those made by adult rats during investigations of conspecifics and during courtship (Barfield & Thomas,

1986). The fact that these high USVs occur not only during the investigation and courtship behaviors of adult rats but also during play solicitation behaviors of juvenile rats supports the notion that these vocalizations generally index an appetitive motivation for social interaction in rats.

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