Anticipation of Rewarding Electrical Brain Stimulation Evokes Ultrasonic Vocalization in Rats

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Adult rats emit increased rates of 50-kHz ultrasonic vocalizations (USVs) before receiving social and pharmacological rewards. This study sought to determine whether anticipation of rewarding electrical stimulation of the brain (ESB) would also elicit these vocalizations. In Experiments 1 and 2, rats showed increased 50-kHz USVs before receiving experimenter-delivered ventral tegmental area (VTA) and lateral hypothalamic (LH) ESB on a fixed time 20-s schedule. In Experiments 3 and 4, rats increased their rate of 50-kHz USVs in response to cues that predicted the opportunity to self-stimulate the VTA or LH. Interestingly, unexpected termination of either type of ESB evoked 20-kHz, rather than 50-kHz, USVs. In Experiment 5, a cue that predicted daily 1-hr feeding sessions increased 50-kHz USVs, whereas a cue that predicted footshock decreased 50-kHz USVs. These effects could not be explained simply by changes in locomotor activity or general arousal. Together, these findings support the hypothesis that short 50-kHz USVs may selectively index a state of reward anticipation in rats.

Ethologists have historically documented that rodents emit ultrasonic vocalizations (USVs) in various biologically relevant settings (e.g., Sales & Pye, 1974). In adult rats, investigators have characterized at least two different types of USV that can be distinguished by a combination of frequency and duration (Miczek, Tornatzky, & Vivian, 1991). Emerging evidence suggests that these two types of USVs also occur in affectively different behavioral contexts.

Rats emit 20-kHz USVs in both social and nonsocial settings. Socially, these vocalizations coincide with submissive behaviors after attack in the context of intermale aggression (Thomas, Takahashi, & Barfield, 1983), the refractory period of males after copulation (Barfield & Geyer, 1972), and isolation from conspecifics (Francis, 1977). Nonsocial stimuli that elicit these vocalizations include predatory odors (Blanchard, Blanchard, Agullana, & Weiss, 1991), anticipation of foot shock (Tonoue, Ashida, Makino, & Hata, 1986), cocaine and morphine withdrawal (Barrios & Miczek, 1996; Mutschler & Miczek, 1998; J. A. Vivian & Miczek, 1991), and electrical stimulation of brain areas implicated in pain perception (Yajima, Hada, & Yoshii,

1976). Although these eliciting stimuli differ, they all have the capacity to evoke either passive or active avoidance behavior in rats. Accordingly, investigators have hypothesized that 20-kHz USVs may reflect negative affective states marked by both high arousal and the anticipation of punishment (Cuomo et al., 1988; Tonoue et al., 1986, but see Portavella, Depaulis, & Vergnes, 1993 for a dissenting view).

Adult and juvenile rats also make a second type of USV called the 50-kHz USV, which, in addition to its higher frequency, also typically has a shorter duration (i.e., less than .3 s; Fu & Brudzynski, 1994). Socially, 50-kHz USVs have been observed during appetitive aspects of sexual behavior (Barfield, Auerbach, Geyer, & McIntosh, 1979), during intermale aggression (mainly by intruders during an initial encounter; Takahashi, Thomas, & Barfield, 1983), and before as well as during juvenile play (Knutson, Burgdorf, & Panksepp, 1998). Nonsocial stimuli that evoke 50-kHz USVs include places associated with prior amphetamine or morphine administration (Knutson, Burgdorf, & Panksepp, 1999) and glutamate stimulation of brain areas associated with sexual behavior (Fu & Brudzynski, 1994). Because 50-kHz USVs co-occur with approach behavior in many of the settings described above, Knutson et al. (1999) hypothesized that in adult rats, they may index a positive affective state akin to excitement that is characterized by high arousal and anticipation of reward.

On the basis of vigorous approach and self-administration behavior it elicits, electrical stimulation of the brain (ESB) in the ventral tegmental area (VTA) and lateral hypothalamus (LH) may serve as a potent reward for rats (Stellar & Stellar, 1985). In this series of experiments, we examined whether experimentally-induced anticipation of rewarding ESB would elicit 50-kHz USVs. We also examined whether

changes in vocalization could be alternatively explained by changes in locomotor activity or nonspecific arousal.

Experiment 1

In Experiment 1, we investigated whether anticipation of VTA ESB would elicit 50-kHz USVs in adult rats. Subjects received single pulses of VTA ESB once every 20 s (FT 20) for 10-min sessions, and both 50- and 20-kHz USVs were recorded during the fixed intervals leading up to ESB administration.

Method

Subjects. Nine adult male Long-Evans rats (300–500 g) bred at the Bowling Green State University animal colony participated in Experiment 1. Subjects were maintained on a 12-hr light–dark cycle (lights on at 8:00 a.m.) and had ad lib access to food and water unless otherwise noted. Colony room temperature was maintained at approximately 23° C.

Preoperative testing. Twenty-four hours before surgery, subjects received 2 min of light "tickling" stimulation to the dorsal nape of the neck by a familiar experimenter. Rats that did not vocalize at least 10 times during one of the 15-s blocks of tickling were excluded (approximately 20%). This was done to ensure that these rats were able to show USVs, as not all adult males exhibit this tendency (Sales & Pye, 1974).

Surgery. Subjects were anesthetized with Nembutal (50 mg/kg ip) and mounted in a Kopf stereotactic instrument (Kopf Instruments, Tujunga, CA). After exposing the skull, bregma and lambda were visualized and aligned on the same horizontal plane. Two holes were drilled 2.0 mm posterior to bregma, each 1.7 mm lateral to the midline on each side. Three additional holes were drilled for insertion of stainless steel self-tapping anchor screws.

Because ESB-induced sniffing in anesthetized rats is positively related to postoperative bar-pressing rates (Rossi & Panksepp, 1992), rats were tested for ESB-induced sniffing during surgery. A 0.5-mm diameter tip, bipolar electrode (insulated except for the cross-sections of the tips) was lowered 7.0 mm below the dura, starting with the left hole, and 100 mA-current ESB was applied for 5 s with a 60-Hz sine wave constant current stimulator (model 82408, Lafayette Instruments, Lafayette, IN). The electrode was then lowered in 0.5 mm increments, and the stimulation was repeated until sniffing was clearly observed or the electrode reached 9.5 mm below dura, in which case the procedure was repeated on the other side of the brain. When a site that elicited clear sniffing was located, the electrode was fixed permanently in place with dental cement.

Training. After a minimum of 2 days of postsurgical recovery, subjects were placed in a 34 × 23 × 32.5 cm translucent box with corn cob bedding on the floor and a 10 × 2 × .25 cm bar elevated 8.5 cm from the floor. Subjects were allowed to bar press for 0.5-s trains of 100-mA ESB on a continuous reinforcement schedule (Rossi & Panksepp, 1992). During this training, subjects received priming pulses and were directed toward the bar as needed. Current intensity was initially set at 20 mA, and was raised by 5 mA every 3 min until vigorous self-stimulation occurred (operationally defined as 90 bar presses/3 min). Each subject's optimal current for eliciting 30 presses per minute was recorded and used in Experiments 1 and 3. Only rats (*n* = 9) that were able to meet the criterion of 90 bar presses/3 min with current intensities of 120 mA or less were included.

Behavioral testing. All behavioral testing occurred under dim lighting (~30 Lux) in a 45 × 17.5 × 20 cm lucite box with corn cob bedding. Subjects received 1-s pulses of VTA ESB at 20 sec intervals on an FT20 schedule for four consecutive daily 10-min test sessions. Although the ESB current was set at each subject's optimal threshold for eliciting self-stimulation during training, ESB administration was not contingent on subjects' behavior during this experiment. Over the course of ESB administration and the intervening intervals, an experimenter recorded 50- and 20-kHz USVs in 5-s bins by using two miniature bat detectors (UltraSound Advice, London, England) mounted 20 cm above the floor of the test chambers. One of the detectors was tuned to 20 kHz, and the other was tuned to 50 kHz. High interrater reliability has been established for this method of coding USVs (Spearman's *r* = .90, *Z* = 5.17, *p* < .0001; Knutson et al., 1998). For purposes of analysis, USVs were averaged across trials within a session. Subjects were tested for four sessions occurring on consecutive days.

Histology. At the conclusion of behavioral testing (i.e., after Experiments 1 and 3), subjects were given an overdose of Nembutal and were transcardially perfused with 200 ml 0.9% saline followed by 500 ml 10% formalin in 0.9% saline. Brains were allowed to postfix for 24 hr in 30% sucrose-10% formaldehyde-0.9% saline solution (w/v). Brains were then frozen and sliced into 80-μm coronal sections with a freezing microtome. Sections through the tips of the electrodes were mounted on microscope slides, stained with cresyl violet, dried, and cover-slipped. All electrode tips were located in the VTA between Plates 39 and 42 as defined by Paxinos & Watson (1997).

Results

USVs were analyzed with 4 (bin-within) × 4 (day-within) repeated measures analyses of variance (ANOVAs). Because no 20-kHz USVs were observed, we report only the results for 50-kHz USVs. As predicted, subjects exhibited systematically increasing 50-kHz USVs in a slightly scalloped fashion over the course of each time bin, until the point of VTA ESB delivery, bin main effect: $F(3, 8) = 15.10$, $p < .0001$ (see Figure 1). This pattern did not change across days, as indicated by the absence of a significant main effect for day and the absence of a significant Bin × Day interaction.
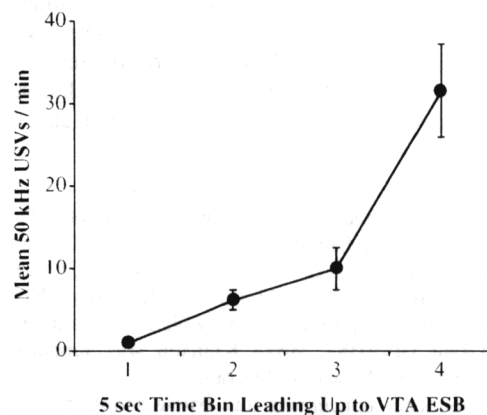

Figure 1. Mean (±*SEM*) 50-kHz ultrasonic vocalizations (USVs) per minute in 5-s time bins leading up to ventral tegmental (VTA) electrical stimulation of the brain (ESB, Day 1 only).

Experiment 2

In Experiment 1, 50-kHz USVs (and not 20-kHz USVs) increased in a scalloped pattern before administration of VTA ESB. However, locomotor activity may also have increased before ESB. According to one theory, rats' 50-kHz USVs result from thoracic compression elicited by the forepaw impact engendered by heightened locomotor activity (Blumberg, 1992). Therefore, in Experiment 2, we measured both USVs and locomotor activity before rewarding ESB to examine whether increased activity could statistically account for increases in vocalization. In addition, we examined whether ESB of the LH would elicit 50-kHz USVs, to test whether our findings would generalize to other brain areas that support self-stimulation.

Method

Subjects. Five adult male Long-Evans rats (300–500 g; Charles River, Wilmington, MA) participated in Experiment 2. Subjects were maintained on a 12-hr light–dark cycle (lights on at 8:00 a.m.) and had ad lib access to food and water unless otherwise noted. Colony room temperature was maintained at approximately 23° C.

Surgery. Subjects were anesthetized with Nembutal (50 mg/kg ip) and mounted in a Kopf stereotactic instrument. After exposing the skull, two holes were drilled 1.8 mm posterior to bregma, each 3.7 mm lateral to the midline on each side. Four additional holes were drilled for insertion of stainless steel self-tapping anchor screws. Bilateral monopolar electrodes (Plastic One, Roanoke, VA) were lowered 8.3 cm below the dura at 10° angles aimed at midline, and the grounding wires were attached to at least one of the anchor screws and secured with dental cement.

Training. Five days after surgery, subjects were placed in a 30 × 25 × 30 cm operant box with a wire mesh floor and two retractable 40 × 20 × 1 cm bars elevated 9 cm from the floor (Med Associates, St. Albans, VT). Subjects were allowed to bar press on a continuous reinforcement schedule for 0.5-s trains of 60 Hz, 0.1-ms anodal square wave ESB. During this training, subjects received priming pulses and were directed towards the bar as needed. Current intensity was initially set at 100 mA, and was raised by 100 mA every 3 min until vigorous self-stimulation occurred (operationally defined as 90 bar presses/3 min). Each rat's optimal current for eliciting 30 presses per minute was recorded and used in Experiments 2 and 4. All 5 rats met the criterion of 90 bar presses/3 min with current intensities of 1000 mA or less. (Note: Differences in current intensities resulted from use of monopolar electrodes with a square wave current in this experiment compared with the bipolar electrodes and sine wave current used in Experiment 1.)

Behavioral testing. All behavioral testing occurred under dim lighting (~30 Lux) in the operant chamber described in the *Method* section of Experiment 1. Subjects received 0.5-s pulses of LH ESB on an FT20 schedule for one 5-min test session. An experimenter used a bat detector (Pettersson Elektronik, Uppsala, Sweden) to record 50- and 20-kHz USVs in 5-s bins leading up to ESB. Quadrant entries (each 15 × 15 cm) were also recorded in 5-s bins. For purposes of analysis, both USVs and quadrant entries were averaged across trials within the session.

Histology. At the conclusion of behavioral testing (i.e., after Experiments 2 and 4), electrode localization was verified as described in Experiment 1. All electrode tips were located in the LH between Plates 26 and 29 as defined by Paxinos & Watson (1986).

Results

USVs and locomotor activity were analyzed with four (bin-within) factor, repeated measures one-way ANOVAs. As in Experiment 1, no 20-kHz USVs were observed, thus we report only the 50-kHz results. As before, subjects exhibited systematically increasing 50-kHz USVs in a scalloped fashion over the course of each time bin, until the point of LH ESB delivery, and this effect remained significant despite covariance for changes in locomotor activity, bin main effect: $F(3, 9) = 27.69, p < .0001$ (see Figure 2). Quadrant entries also increased in the same pattern; however, covariance for changes in vocalization eliminated the significance of this effect, bin main effect: $F(3, 9) = 2.87$, $p = .10$. These results suggest that although locomotor activity also increased before stimulation, changes in gross locomotor activity could not simply account for increases in 50-kHz USVs.

Experiment 3

In Experiments 1 and 2, rats selectively increased their rate of 50-kHz USVs in anticipation of experimenter-delivered pulses of VTA and LH stimulation. However, because we did not allow the rats to self-administer the stimulation during testing, it may have also carried aversive characteristics. In this experiment, we examined the ability of a cue associated with self-administered VTA ESB to evoke 50-kHz USVs. To achieve this, we passively exposed subjects to a self-stimulation cue by separating them from the self-stimulation bar with a wire mesh screen. To control for the possibility that this separation might induce "frustrative nonreward" instead of reward anticipation (Hug & Amsel, 1969), we contrasted vocalizations during this cue exposure condition with vocalizations during extinction of bar pressing.

Method

Subjects. The 9 adult male Long-Evans rats described in Experiment 1 also participated in this experiment.
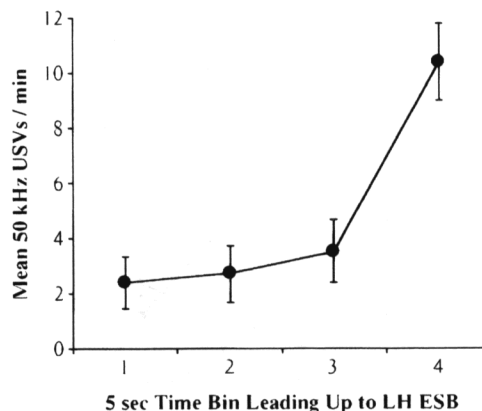

Figure 2. Mean (±*SEM*) 50-kHz ultrasonic vocalizations (USVs) per minute in 5-s time bins leading up to lateral hypothalamic (LH) electrical stimulation of the brain (ESB).

Training. After completing Experiment 1, subjects were reacclimated to bar pressing on a continuous reinforcement schedule for 0.5-s trains of ESB during a single 10-min session.

Behavioral testing. Testing occurred under dim lighting (~30 Lux) in an operant box as described in Experiment 2. Behavioral testing consisted of two identical bar-pressing sessions on subsequent days, with modified periods appended to the end of each session. On Day 1, immediately after the 10-min bar-pressing session, a 25 × 30 cm wire mesh screen that blocked subjects' access to the bar was placed in the middle of the chamber, so that subjects could passively view, but not press, the self-stimulation bar for an additional 2 min (the "cue exposure" period). On Day 2, immediately after the 10-min bar-pressing session, subjects were not placed behind the screen, but rather were placed on extinction for 2 min, so that they could press the bar, but it would no longer deliver stimulation. These manipulations were not counterbalanced so as to avoid "contaminating" the cue exposure period with expectations of nonreward from previous extinction periods. Fifty- and 20-kHz USVs were recorded for six 2-min blocks spanning bar pressing and the manipulations.

Results

Paired *t* tests were used to compare 50- and 20-kHz USVs across cue exposure and extinction periods. As predicted, a significant effect indicated that cue exposure elicited more 50-kHz USVs than did extinction, $t(8) = 5.48, p < .001$ (see Figure 3). Interestingly, a second test indicated that the manipulation had the opposite effect on 20-kHz USVs. Specifically, extinction produced more 20-kHz USVs than did cue exposure, which evoked none, $t(8) = -5.49, p < .001$. These results suggested not only that anticipation of self-administered LH ESB can evoke 50-kHz USVs, but also that this effect is not due to induction of frustrative nonreward by extinction.

Experiment 4

The results of Experiment 3 further supported the hypothesis that cues associated with rewarding ESB can evoke 50-kHz USVs, as passive exposure to the self-stimulation bar evoked higher rates of 50-kHz USVs than did extinction. Although extinction clearly should reduce anticipation of
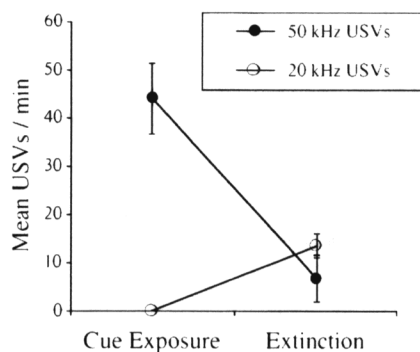
reward, the ability of the cue exposure manipulation to increase anticipation of reward could be questioned because subjects had not been trained to associate cue exposure with future opportunities for self-administering VTA ESB. Thus, in Experiment 4, we trained subjects to associate a light cue with subsequent self-administration of LH ESB and measured USVs during presentation of the cue.

In addition, although the cue exposure manipulation in Experiment 3 precluded rats from pressing the bar, and so reduced their activity, we did not concurrently measure other types of activity during this manipulation. Therefore, in this experiment, we concurrently recorded locomotor activity during cue presentation to ensure that changes in vocalization could not be accounted for by changes in activity.

Method

Subjects. The 5 adult male Long-Evans rats described in Experiment 2 also participated in this experiment.

Behavioral testing. Testing occurred in a dimly lit operant box as described in Experiment 2. Subjects received the opportunity to self-stimulate with LH ESB for 1-hr sessions on 3 consecutive days. Two minutes before the start of each session, a cue light was turned on, and it remained on until the session began. At the beginning of the session, the cue light turned off, the normal lighting returned, the retractable active and passive levers extended into the box, and subjects were allowed to self-administer LH ESB on a continuous reinforcement schedule. On the final (third) day of testing, the stimulator was turned off 2 min after the start of self-administration session, so that subsequent barpresses no longer delivered VTA ESB. Bar presses were recorded by a personal computer (WMPC, 1999). USVs and quadrant entries were recorded by an on-line coder with a bat detector in 2 min blocks throughout testing.

Results

Fifty- and 20-kHz USVs during cue exposure were examined across the 3 days with one-way ANOVAs. Quadrant entries during cue exposure were also examined across the 3 days with a one-way ANOVA. Because no 20-kHz USVs were observed during cue exposure, we again present only the 50-kHz results (see Figure 4). A significant main effect indicated that 50 kHz USVs increased over days, $F(2, 6) = 11.54, p < .01$ after covarying for changes in activity. Tukey's honestly significant difference post hoc pairwise comparisons indicated that 50-kHz USVs were greater on Days 2–3 than on Day 1 ($p < .01$). However, activity during cue exposure did not change significantly over days either before or after covarying for changes in vocalizations. As in Experiment 2, these results suggest that cue-induced increases in 50-kHz USVs cannot be accounted for by increases in locomotor activity.

Fifty- and 20-kHz USVs were also compared across the final day of cue exposure and the extinction period with paired *t* tests. As in Experiment 3, cue exposure elicited significantly more 50-kHz USVs than did extinction, $t(4) = 7.84, p < .005$, whereas extinction evoked more 20-kHz USVs than did cue exposure, which by itself produced none, $t(4) = -4.89, p < .01$ (see Figure 5). As with 50-kHz USVs, paired *t* tests indicated that quadrant entries also were lower

Figure 3. Mean (±*SEM*) ultrasonic vocalizations (USVs) per minute during 2-min cue exposure associated with ventral tegmental (VTA) self-administered electrical brain stimulation versus extinction of VTA self-stimulation.

during extinction than during cue exposure (i.e., 8.4 ± 1.0 vs. 17.0 ± 2.0) $t(4) = 4.12$, $p < .05$. However, this result probably reflected the fact that rats spent more time pressing the bar during extinction (19.8 ± 4.1) than during cue exposure, when they did not press the bar at all. Once again, anticipation of rewarding ESB, but not frustration, elicited 50-kHz USVs.

Experiment 5

The experiments described above suggested that cues for both experimenter- and self-administered rewarding ESB can evoke 50-kHz USVs. If these vocalizations indeed index a state of reward anticipation, then cues for natural rewards should also elicit them. However, if 50-kHz USVs instead index a state of general arousal, then cues for punishment should have the capacity to evoke them. In this experiment, we sought to validate 50-kHz USVs as an index of reward anticipation by examining whether cues for food availability would increase their expression and whether cues for footshock would decrease them.
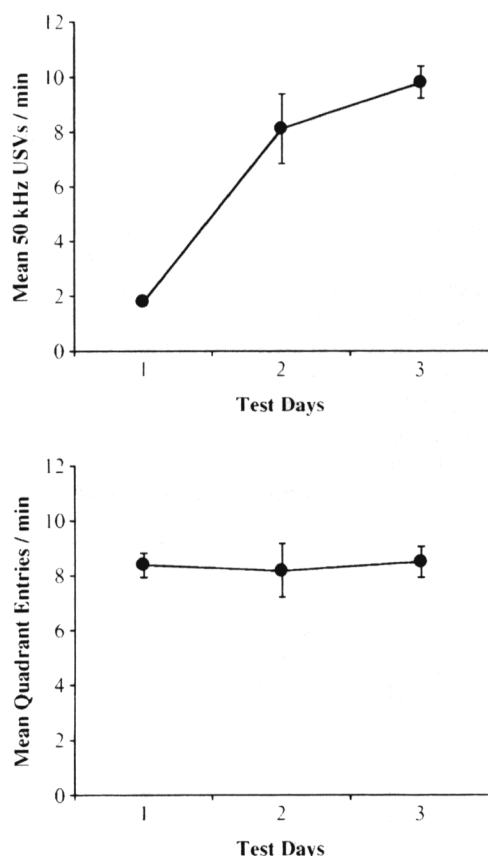

Figure 4. Mean (±SEM) 50-kHz ultrasonic vocalizations (USVs, top panel) and quadrant entries (bottom panel) per minute during 2-min cue exposure that predicted self-administered lateral hypothalamic electrical brain stimulation.
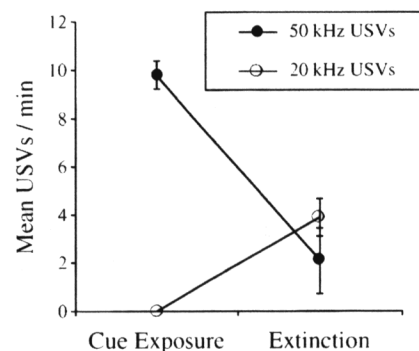

Figure 5. Mean (±SEM) ultrasonic vocalizations (USVs) per minute during 2-min cue exposure that predicted self-administered lateral hypothalamic (LH) brain stimulation versus extinction of LH self-stimulation.

Method

Subjects. Eight adult male Long-Evans rats (300–500 g) participated in this experiment. Subjects were maintained on a 12-hr light–dark cycle (lights on at 8:00 a.m.). Colony room temperature was maintained at approximately 23° C.

Behavioral testing. Testing occurred in subjects' home cages, which were 30 × 20 × 30 cm boxes with stainless steel rod flooring. For 6 consecutive days, subjects were allowed only 1 hr of access to food (standard Purina Rat Chow) per day. Two minutes before the start of each daily feeding session, a white light situated 20 cm above the floor was turned on, and it stayed on ("cue exposure") until the beginning of the feeding session, when subjects received a hopper full of chow. On the 7th day, instead of receiving food, an empty hopper was placed in subjects' cages ("extinction"). USVs, quadrant entries, and latency to eat were recorded by an on-line coder during the cue exposure and extinction periods, and amount of food eaten was recorded at the end of each daily feeding session.

After the 7th day of testing, subjects were given ad lib access to food for 3 days until they regained their baseline weight. Then, for 5 more days, the same cue light was illuminated for 2 min, followed by a single 0.5-s scrambled footshock. Again, USVs and quadrant entries were recorded by an on-line coder during cue exposure periods.

Results

Changes in 50- and 20-kHz USVs were examined across the 6 days of food cue exposure with one-way ANOVAs. Quadrant entries during food cue exposure were similarly analyzed (see Figure 6, a and c). As before, no 20-kHz USVs were observed during cue exposure. A significant main effect indicated that 50-kHz USVs increased over days, $F(5, 30) = 11.12$, $p < .0001$ after covarying for changes in activity. Post hoc pairwise comparisons indicated that 50-kHz USVs were greater on Days 5–6 than on Days 1–4 ($p < .001$). Activity during cue exposure also increased over days, $F(5, 30) = 2.85$, $p < .05$ after covarying for changes in USVs, and post hoc analyses indicated that activity was greater on Days 2–6 than on Day 1 ($p < .005$). Once again, in spite of the fact that both 50-kHz USVs and activity increased over days, they seemed to follow different
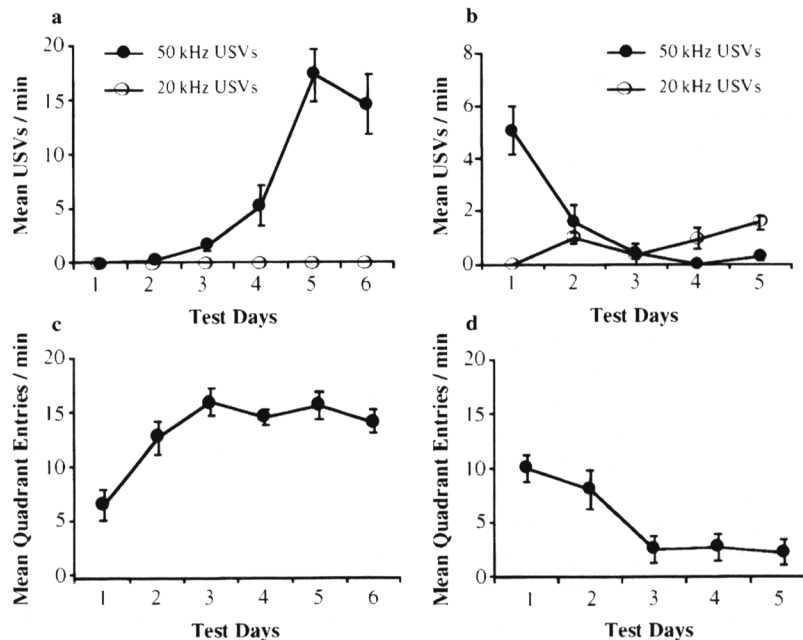
Figure 6. Mean (±*SEM*) ultrasonic vocalizations (USVs, a and b) and quadrant entries (c and d) per minute during 2-min cue exposure that predicted food availability (a, c) and 2-min cue exposure that predicted footshock (b, d).

patterns, such that changes in cue-induced activity could not account for increases in 50-kHz USVs.

One-way ANOVAs were also performed on USVs and activity across the 5 days of shock cue exposure (see Figure 6, b and d). Fifty-kHz USVs decreased over days, $F(4, 24) = 6.29, p < .01$ after covarying for activity. Post hoc pairwise comparisons indicated that they dropped immediately after the first day of shock exposure, as Days 2–5 showed significantly fewer USVs than Day 1 ($ps < .001$). Activity also decreased, $F(4, 24) = 3.62, p < .05$ after covarying for 50-kHz USVs, but persisted longer, as post hoc pairwise comparisons indicated that activity levels were significantly lower on Days 4–5 than on Days 1–3 ($ps < .005$). This reduction in activity was probably a function of the freezing behavior eventually observed in 7 of the 8 subjects tested.

Finally, 50- and 20-kHz USVs were compared across the last food cue exposure period and subsequent extinction period (i.e., presentation of empty food bin) with paired t tests. As in previous experiments, food cue exposure elicited significantly more 50-kHz USVs than did extinction, $t(7) = 5.26, p < .005$, whereas extinction produced more 20-kHz USVs than did cue exposure, which by itself produced none, $t(4) = -4.07, p < .01$ (see Figure 7). Thus, the effects found with rewarding ESB appeared to generalize to a more naturalistic rewarding stimulus such as food.

Discussion

This work provides the first demonstration that anticipation of rewarding brain stimulation can elicit USVs in rats. In Experiments 1 and 2, rats increased their rate of 50-kHz

USVs immediately before receiving experimenter-delivered, fixed-interval pulses of electrical stimulation to either the VTA or LH. In Experiments 3 and 4, cues that signaled the opportunity to self-stimulate also elicited 50-kHz USVs. These patterns held for natural rewards as well, as a cue that predicted food availability increased 50-kHz USVs in Experiment 5. Together, these findings seem to indicate that anticipation of rewarding brain stimulation can selectively elicit 50-kHz USVs in rats. However, before this hypothesis can be accepted, several alternatives must be addressed.

One alternative explanation for these findings might hold that 50-kHz USVs are simply a physiological byproduct of increased motor activity (e.g., Blumberg, 1992; Thiessen, Kittrell, & Graham, 1980). Indeed, in two of three experiments in which we measured both USVs and activity, we
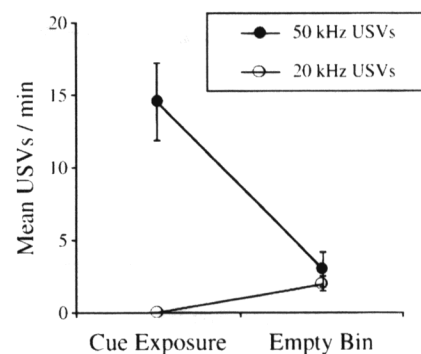

Figure 7. Mean (±*SEM*) ultrasonic vocalizations (USVs) per minute during 2-min cue exposure for food delivery versus presentation of the empty food bin.

observed that both 50-kHz USVs and activity increased on presentation of reward cues. However, closer inspection revealed that changes in activity could not account for increases in vocalization. First, covariance analysis demonstrated that increases in activity could not statistically account for increases in 50-kHz USVs before experimenter-administered LH ESB, whereas changes in vocalizations could statistically account for changes in activity (Experiment 2). Second, rats increased their rate of 50-kHz USVs over days as they learned to associate a cue light with subsequent opportunities for self-administering LH ESB, whereas activity did not change significantly (Experiment 4). Finally, cues for natural rewards and punishments had differing effects on activity and 50-kHz USVs. Rats increased their rate of activity before increasing their rate of 50-kHz USVs as they learned to associate a cue light with subsequent food availability. Conversely, 50-kHz USVs dropped long before activity diminished as rats later learned to associate the same cue with foot shock (Experiment 5). These dissociations suggest that the expression of 50-kHz USVs do not depend on gross locomotor activity, although certain types of locomotor activity may of course be more tightly linked to these vocalizations than others.

According to a second alternative explanation, 50-kHz USVs might index a state of general or unvalenced arousal, rather than anticipation of reward. On the basis of this inference, one could extrapolate that cues that invoke anticipation of punishment should also increase 50-kHz USVs. However, several findings suggest otherwise. Presentation of cues for self-administered VTA ESB, LH ESB, and food all selectively elicited 50-kHz but not 20-kHz USVs (Experiments 3–5). On the other hand, others have reported that presentation of shock cues increases 20-kHz USVs (Tonoue et al., 1986). We replicated this effect and further found that presentation of shock cues decreased 50-kHz USVs rather than increasing them (Experiment 5). A general arousal explanation of USV production cannot parsimoniously account for the fact that reward cues selectively elicit 50-kHz but not 20-kHz USVs, whereas punishment cues have the opposite effect.

Interestingly, we also observed that extinction evokes 20-kHz USVs. This might accord with an extensive body of literature suggesting that the 20-kHz USV primarily indexes negative affective states (reviewed in Miczek, Weerts, Vivian, & Barros, 1995). Even though unexpected termination of rewarding ESB operationally represents a reward decrement, animals may perceive this sudden shift in contingencies as more of an increment in punishment. A similar affective dynamic may underlie the production of 20-kHz USVs during cocaine and morphine withdrawal (Mutschler & Miczek, 1998; Vivian & Miczek, 1991).

Although our current results appear to unambiguously support the hypothesis that 50-kHz USVs mark a state of reward anticipation in rats, some ethological studies seem to indicate that this vocalization may also serve other functions. Certainly, in diverse rewarding social encounters including sex (Barfield et al., 1979) and play (Knutson et al., 1998), 50-kHz USVs not only occur during approach behaviors but also in anticipation of such behavior. How-ever, 50-kHz USVs also occur in the context of less obviously rewarding social interactions such as intermale aggression (Vivian & Miczek, 1993), though some have argued that rats may find certain aspects of aggression rewarding (Taylor, 1976). Future component analyses which examine the fine-grained associations between approach versus avoidance behaviors and 50-kHz USVs in complex social interactions can better establish whether our predictions generalize to most ethological settings. For instance, in the context of intermale aggression, behaviors associated with passive or active avoidance are almost always accompanied by 20-kHz USVs rather than 50-kHz USVs (Portavella et al., 1993).

In sum, these findings indicate that internal or external cues that predict rewarding brain stimulation increase the expression of 50-kHz USVs in adult rats. In addition to supporting the hypothesis that 50-kHz USVs mark a state of reward anticipation, these findings imply that USVs may serve as useful tools for distinguishing different types of affective states in rats.

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