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Authors

Barfield, RJ
Geyer, LA

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THE ULTRASONIC POSTEJACULATORY VOCALIZATION AND THE POSTEJACULATORY REFRACTORY PERIOD OF THE MALE RAT[1]

RONALD J. BARFIELD[2] AND LYNETTE A. GEYER

Department of Biology, Livingston College, Rutgers—The State University

After ejaculation, the male rat emits an ultrasonic (22-kHz.) vocalization. This sound is produced repeatedly until about three fourths of the postejaculatory interval has elapsed. In this study, the occurrence of the vocalization was described, and physiological and behavioral evidence was presented that the postejaculatory vocalization reflects an inhibitory state that underlies the postejaculatory refractory period. The vocalization period was characterized by a predominance of slow-wave, spindling, sleeplike electroencephalographic activity. Electrical shock was able to stimulate mating responses only after the cessation of the vocalization period. It was concluded that an *absolute* refractory period of the postejaculatory interval lasts until the end of the vocalization period and that the time from the termination of the vocalization until the resumption of mating is a *relative* refractory period.

After the male rat ejaculates, he enters a period of copulatory refractoriness. During the first part of this period, the male shows little movement and, at times, maintains a tonic immobility (Dewsbury, 1967). Electroencephalographic (EEG) recordings during this period yield a predominance of irregular high-amplitude, slow-wave, and spindling activity from the hippocampus as well as cortical spindling (Kurtz & Adler, 1973); these records are characteristic of an inhibited, sleeplike state (Lindsley, 1960).

Beach and Holz-Tucker (1949) characterized 2 phases of the postejaculatory refractory period, an absolute and a relative refractory period. During the absolute phase, the rat is incapable of sexual response, but the relative phase is labile and is influenced by the arousability of the male and the nature of the stimulus situation. Until recently, however, there was no means by which these 2 periods could be objectively measured. Studies on shock-induced copulatory behavior showed that the total refractory period, the postejaculatory interval, could be reduced to about 75% of its control value by the administration of periodic .5-sec. shocks to the skin (Barfield & Sachs, 1968). On the basis of that study, Sachs and Barfield (1974) proposed that the absolute refractory period was that time during which shocks were ineffective and that the relative refractory period was virtually eliminated by the treatment.

Subsequently, it was discovered that during the refractory period males emit an ultrasonic (22-kHz.) vocalization (Barfield & Geyer, 1972), and we proposed that this emission was an acoustical concomitant of the absolute refractory period since it lasted about 75% of the postejaculatory interval. That is, the vocalizations terminate at about the time that shocks would be expected to become effective in rearousing copulatory behavior.

Except for shock, or handling in old rats (Larsson, 1963), stimulus variations have little effect on the length of the refractory period, i.e., the postejaculatory interval (PEI). Presentation of a novel female can shorten the refractory period dramatically, but only after the males are sexually ex-

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[2] Requests for reprints should be sent to R. J. Barfield, Department of Biology, Livingston College, Rutgers University, New Brunswick, New Jersey 08903.

hausted and do not spontaneously reinitiate mating (Fisher, 1962; Wilson, Kuehn, & Beach, 1963). Hsiao (1965) has demonstrated that changing the stimulus female after ejaculation but prior to exhaustion had no effect on the length of the refractory period; however, it was not known whether different components of the period might be differentially affected by the change in stimulus.

The objective of the present studies was to present physiological and behavioral evidence in support of the hypothesis that the postejaculatory vocalization is a concomitant of the absolute refractory period. The specific goals were: (*a*) to describe the postejaculatory vocalization and the associated behavior of the male and female during normal mating sequences, (*b*) to determine whether there is a relationship between the slow-wave, sleeplike EEG of the refractory male and the emission of 22-kHz. vocalizations, (*c*) to determine whether the male is responsive to the facilitative effects of shock during the vocalization period, and (*d*) to determine whether change of the stimulus female prior to exhaustion affects either component of the refractory period.

Experiments 1 and 2

Method

Subjects. Intact adult male Long-Evans hooded rats that had previously been determined to be reliable maters were used. Stimulus subjects were ovariectomized Long-Evans and Sprague-Dawley females. The females either were treated with 33- or 66-μg. estradiol benzoate in peanut oil 54 hr. prior to testing or had a chronic subcutaneous estradiol benzoate pellet implant. All females were injected with 500-μg. progesterone in peanut oil 4–6 hr. prior to testing.

All animals were housed in 30 × 18 × 18 cm. cages with food and water available continuously. They were kept under a semireversed lighting schedule (lights off 10:30 a.m.–10:30 p.m.).

Ultrasound monitoring. Ultrasounds were monitored by a Holgate ultrasonic receiver. This is a superheterodyne instrument that produces an audible output in response to ultrasonic input. The instrument tunes to a bandwidth of approximately 5 kHz. so that selection of a frequency range to be monitored is possible. The preamplified microphone output can be simultaneously monitored directly on an oscilloscope so that any ultrasound detected by the microphone transducer will be displayed. In practice, all ultrasonic emissions heard on the receiver were confirmed by the presence of a visible waveform of the appropriate frequency on the oscilloscope screen.

Electroencephalographic recording. All EEGs were recorded from chronically implanted hippocampal and cortical electrodes. Animals were anesthetized and placed in a stereotaxic instrument; deep (dorsal hippocampal) electrodes were lowered into place according to the stereotaxic atlas of Pellegrino and Cushman (1967). Cortical electrodes were No. 80 stainless steel screws. Both bipolar and unipolar hippocampal electrodes were used. Electrodes were attached to the skull with Caulk Grip dental cement and were connected to an ITT Micro-D miniature connector. The entire assembly was secured to the skull with dental acrylic. A shielded flexible connector cable (Caltron Industries) attached to a Micro-D plug linked the animal to a Grass Model 79 polygraph. This arrangement allowed continuous recordings from freely moving animals to be made with only minimal movement artifact. Cortical EEG recordings were made with filters set at 10 and 75 Hz. For hippocampal recordings, filters were set at 1 and 15 or 35 Hz.

Ultrasounds were simultaneously recorded by feeding the speaker output of the heterodyne receiver into the integrator channel of the polygraph. With appropriate adjustment, the low-voltage input was transduced into a sharply defined on/off trace that faithfully followed the audible signal from the detector.

Electrical shock. Skin electrodes made from sharpened common safety pins soldered to spring-loaded insulated wire leads were attached to the skin of the flanks on opposite sides of the body. The leads were connected to an electric commutator. The shock current was supplied by a Grass S-8 stimulator through a Grass constant current unit. A sensitive relay in series with the shock current operated a remote switch on a strip chart event recorder so that the delivery of the shock was automatically recorded on the same record as the observed behavior.

Shock intensities that caused animals to startle and emit a small squeal were adopted. These were always between 1 and 3 ma. Shock pulses were 5 msec. and were delivered at a frequency of 90 per second in pulse trains .5 sec. long, either automatically every 30 sec. throughout the tests or by manual control at designated times according to experimental protocol.

Testing procedure. All tests were carried out between 3 and 7 p.m. under red illumination. Males were placed in the test cage, a 10-gal. aquarium (40 × 26 × 29 cm.) with cedar shavings on the floor, 5 min. before the introduction of the female. The following items of sexual behavior were recorded on a push-button actuated stripchart event recorder: mounts with pelvic thrusting, mounts with penile insertion (intromission), ejaculation, and the occurrence of 22-kHz. vocalizations. Only one male was tested at any one time. Testing continued until the first intromission following the

third ejaculation. Males were tested at intervals of 1 wk.

Behavioral measures. Data analyzed were expressed in terms of the following measures: intromission frequency (IF = the number of intromissions prior to ejaculation), ejaculatory latency (EL = time from the first intromission to ejaculation), vocalization latency (VL = time from ejaculation until the beginning of 22-kHz. vocalization), vocalization termination (VT = time from ejaculation until the end of 22-kHz. vocalizations), and postejaculatory interval (PEI = time from ejaculation until the first intromission of the next copulatory series). Two derived measures were also used: PEI minus VT and the ratio VT/PEI.

Results

Experiment 1: A description of the behavior. When the male rat dismounts from the female following ejaculation, it generally grooms its genitalia for a number of seconds, shuffles about, and lies down. Within 35 sec. after the first ejaculation, it begins to emit 22-kHz. sounds in a regular and repeated fashion. Often these vocalizations begin even while the male is still grooming or moving about. The sounds are emitted almost continuously thereafter until about 75% of the postejaculatory interval has elapsed. Toward the end of the vocalization period, the male often begins to emerge from its sedate state, move about, and groom. Often the vocalizations continue into this transitional period, but they become more irregular and are, at times, disrupted by the movements of the paws over the mouth and nose. At other times, however, the male arises from the lying posture, and vocalizations cease immediately. After cessation of the vocalization, the male generally grooms, walks about for 1–2 min., and then reinitiates mating activity. In a few cases, males have begun to mate within seconds of the termination of vocalization, but this was infrequent. With each succeeding ejaculation, the pattern is much the same, although as the successive refractory periods grow longer, the periods of vocalization also increase. A quantitative account of parameters of the postejaculatory refractory period over the course of 3 ejaculatory series is presented in Table 1. Over the course of 3 ejaculatory series, postejaculatory interval (PEI), vocalization termination (VT), and PEI − VT all increase significantly ($p < .025$).

TABLE 1

TEMPORAL PARAMETERS OF THE POSTEJACULATORY VOCALIZATION AND OTHER MEASURES OF MALE RAT COPULATORY BEHAVIOR

Behavioral measure	Ejaculatory series						$F(df = 2/14)$
	1		2		3		
	M	± *SE*	*M*	± *SE*	*M*	± *SE*	
Intromission latency (IL)	174.6	45.6					
Intromission frequency (IF)	11.1	1.5	5.2	.50	6.4	.86	6.23**
Ejaculatory latency (EL)	859	121	337	33.8	440	88	11.43***
Vocalization latency (VL)	36.0	4.0	42.8	10.2	49.2	13.0	.53
Vocalization termination (VT)	328	14.9	415	19.0	427	33.4	4.49*
Postejaculatory interval (PEI)	429	18.9	528	19.9	619	34.3	16.19***
PEI − VT	101	15.1	117	18.5	191.4	28.4	8.52**
Ratio: VT/PEI	.77	.026	.79	.028	.69	.042	3.72*

Note. All means are in seconds. $n = 8$, two tests each. Data were pooled from the two tests.

* $p < .05$.

** $p < .025$.

*** $p < .001$.

Latency to vocalize (VL) does not appear to change, but the ratio VT/PEI decreases significantly ($p < .05$).

While vocalizing, the animals exhibit an unmistakable, irregular pattern of breathing. Sound is emitted during 1–3 sec. periods of expiration and is interrupted by .25-sec. periods of inspiration. After ejaculation, or at times, following intromission, females are observed to emit the same frequency vocalization for several seconds; this is usually associated with a defensive attitude and may be caused by painful stimuli resulting from the copulation. We have also observed a male emitting brief 22-kHz. vocalizations during the final two thirds of the period preceding ejaculation. Out of more than 60 rats that we have observed this was seen in only 2 or 3, and this was characteristic of their performance.

The sound is almost a pure tone, 22–23 kHz., and is emitted in pulses 1–3 sec. long. There is only a minimal modulation of frequency, but a good deal of amplitude change. Vocalizations are low in intensity during the beginning and end of the vocalization period and are loudest during the time that the male lies in an immobilized state on the substratum. We have previously measured the intensity to be as high as 80 db. (SPL; Barfield & Geyer, 1972).

While the male lies still and vocalizes, the female is also often quiescent. The female appears attentive to the male at times, but each tends to remain at opposite ends of the mating cage. The more highly estrous females appear to be the most attentive to the males' calls in this way, and when the vocalizations terminate, these females often dramatically initiate solicitation behavior even before the male regains responsiveness.

Experiment 2: EEG correlates of the post-ejaculatory vocalization. EEG recordings were obtained from 4 males during copulatory behavior. Hippocampal records were classified as (*a*) rhythmical slow activity (theta), (*b*) irregular low-amplitude activity, or (*c*) high-amplitude irregular activity (slow-wave, sleeplike activity; Vanderwolf, 1969). Cortical records were scrutinized for the presence of high-voltage, slow-wave, spindling activity (Klemm, 1969).

During the period prior to the initiation of the vocalization, the males were generally active, usually groomed their genitalia, and EEGs characteristic of an alert, awake animal were recorded. When the males became quiescent and began to vocalize, the development of slow-wave, sleeplike EEG was apparent. As the refractory periods progressed, the spindling pattern became more pronounced in both hippocampal and cortical traces (see Figure 1).

At the termination of the vocalization period, there was, at times, an abrupt change from a sleeplike EEG to an alert pattern (low-amplitude irregular or theta rhythm), and the animals began some activity such as walking, looking about, sniffing, or grooming (see Figure 2). At other times, sleeplike activity persisted beyond the termination of vocalization when the males lay quietly, not obviously changing their overt behavior. Finally, the males often emitted sporadic bursts of vocalization at the termination of the vocalization period. Correspondingly, their EEGs were observed to vacillate between sleeplike and aroused patterns as they shifted from inactivity to activity (see Figure 3).

In the present observations, 22-kHz. vocalizations were accompanied by sleeplike EEG patterns most of the time. Slow-wave patterns occurred independently of vocalization. Thus, one cannot assume that calls are emitted whenever sleeplike activity occurs; however, the converse is almost always true. In general, as shown in Table 2, an alert EEG is characteristic of the vocalization latency period, a sleeplike EEG is observed throughout the vocalization period, and the final segment of the refractory period is characterized by an alert EEG.

Experiment 3

If the vocalization period is, in fact, the absolute refractory period and if electric shock abbreviates the PEI to the limits of the absolute period, then the period of vocalization in the ad-lib condition should be approximately equivalent to the PEI under the shock condition, and shocks before termination of vocalization should not stimulate mating. The present experiment was under-

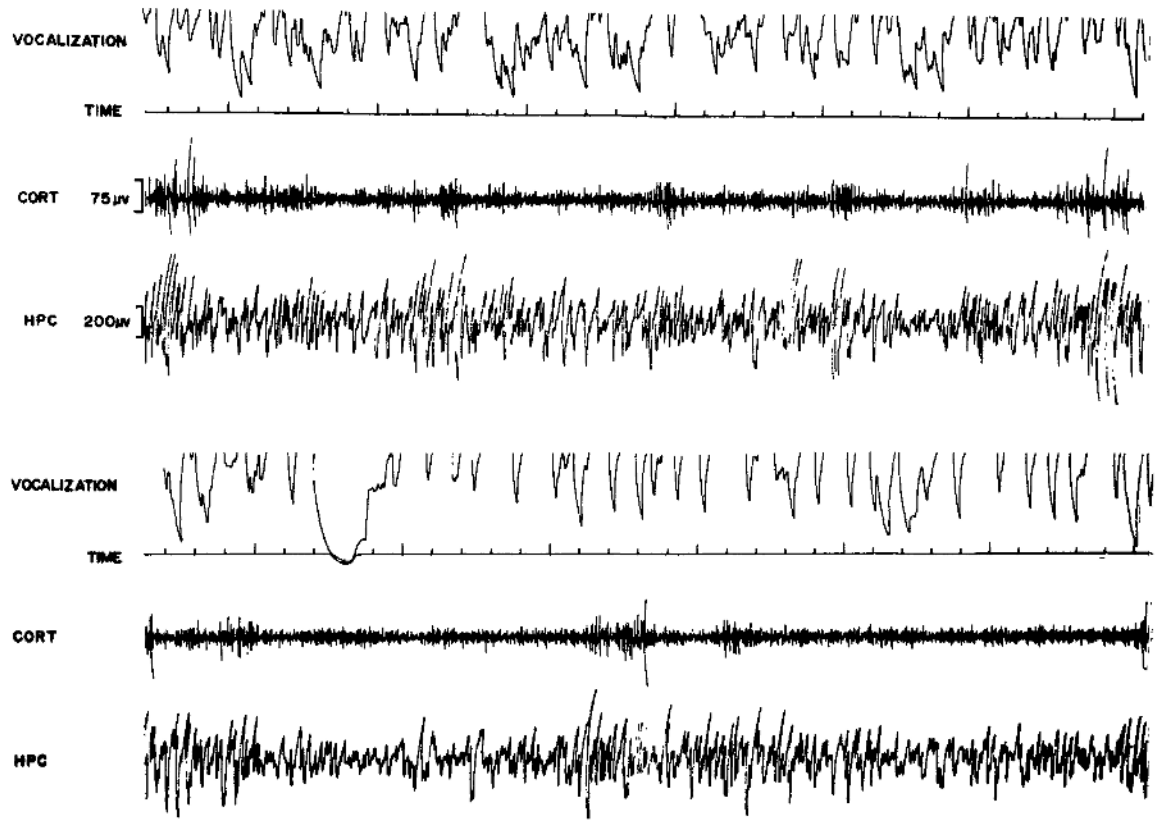

FIGURE 1. Cortical (CORT) and hippocampal (HPC) electroencephalograms during persistent 22-kHz. vocalization. (Vocalization traces represent pulses of sound. Traces are on/off only; where tops are clipped off, sound pulses continued. Dips between pulses represent intervals between vocalizations. When the vocalization trace is flat, no sounds were emitted. Time marker is in seconds.)

taken to test this, but with the additional test condition of a novel stimulus female introduced after each ejaculation. Although the "Coolidge effect" has never been shown to be effective prior to sexual exhaustion, it was quite possible that, as Beach and Holz-Tucker (1949) suggested, a fresh female might reduce the relative refractory period.

Method

Eight intact Long-Evans males were used as subjects. Each was tested 6 times, twice in each of 3 conditions in the following repeated order: (*a*) ad lib, (*b*) electrical shock delivered every 30 sec., and (*c*) a new stimulus female supplied 1 min. after each ejaculation. Tests occurred at 1-wk. intervals and lasted until the first intromission following the third ejaculation. Data were subjected to an analysis of variance appropriate to the $8 \times 3 \times 3 \times 2$ design.

Results

The results are summarized in Table 3 and Figure 4. The period PEI − VT, the hypothetical relative refractory period, was significantly shortened by shock ($p < .001$), as predicted, but the presentation of a novel female did not reduce this period. The effect of shock was most clear in the first PEI. Mating was reinstated about 30 sec. following cessation of the 22-kHz. vocalization; since shocks were delivered every 30 sec., this represents an average shock-to-mount latency of 15 sec. in the first PEI. At no time were shocks seen to induce mating before termination of the vocalizations. Without shock, in the other conditions, mating was not reinstated until an average of 100 sec. after vocalization termination.

Shock resulted in shorter PEIs than in the control and changed-female conditions, but the prediction that PEI shock would be equal to VT ad lib was not borne out because of the unexpected finding that shock increased the time of termination of vocalization, although this effect was not quite

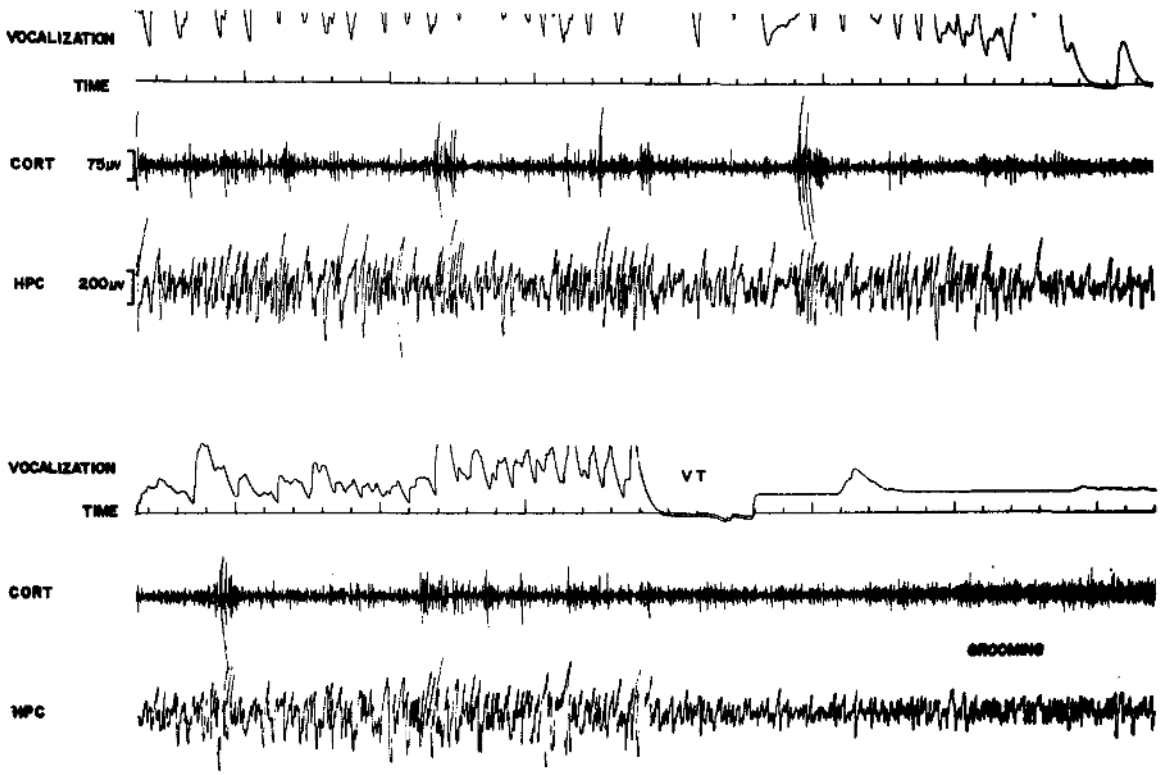

FIGURE 2. Cortical (CORT) and hippocampal (HPC) electroencephalograms during the period of termination of vocalization. (In this case, there was an abrupt shift to an alert, active state. Vocalization traces represent pulses of sound. Traces are on/off only; where tops are clipped off, sound pulses continued. Dips between pulses represent intervals between vocalizations. When the vocalization trace is flat, no sounds were emitted. Time marker is in seconds. Overt behavior is indicated on record. Abbreviation: VT = termination of vocalization.)

statistically significant ($.05 < p < .10$). Application of electrical shock increased the ratio VT/PEI ($p < .001$) and decreased both the latency to vocalize ($p < .05$) and ejaculation latency ($p < .025$).

Over the 3 copulatory series, VT, PEI, and PEI − VT all increased significantly ($p < .01$). The ratio VT/PEI decreased ($p < .05$), and VL was not significantly affected.

EXPERIMENT 4

The results of the previous experiment were confounded by the unexpected finding that repeated shocks lengthened the vocalization period. It was, therefore, necessary to provide shock only after vocalization had spontaneously terminated in order to test the hypothesis that males are capable of copulation when the postejaculatory vocalization ceases. Painful stimuli themselves can induce 22-kHz. vocalization (Barfield & Geyer, 1972), and in the previous experiment, it was observed that shocks reinitiated vocalizations at times.

Method

In the present experiment, 8 intact Long-Evans males were used. Each was exposed once to each of 3 experimental treatments in a random sequence: (*a*) ad-lib copulation, (*b*) preshock (to set shock level) and wires attached, and (*c*) preshock and a single shock (.5 sec.) delivered within 10 sec. of the spontaneous cessation of vocalization. All other procedures were as described previously. An analysis of variance was performed on the data.

Results

When a single shock was delivered at the time the males stopped vocalizing, mating was reinitiated after only a brief delay. Table 4 shows that PEI − VT was reduced to an average of 36 sec. or less ($p < .025$); however, shocks were not delivered immediately on cessation of vocalization. Generally, 10–15 sec. were allowed before the shock to

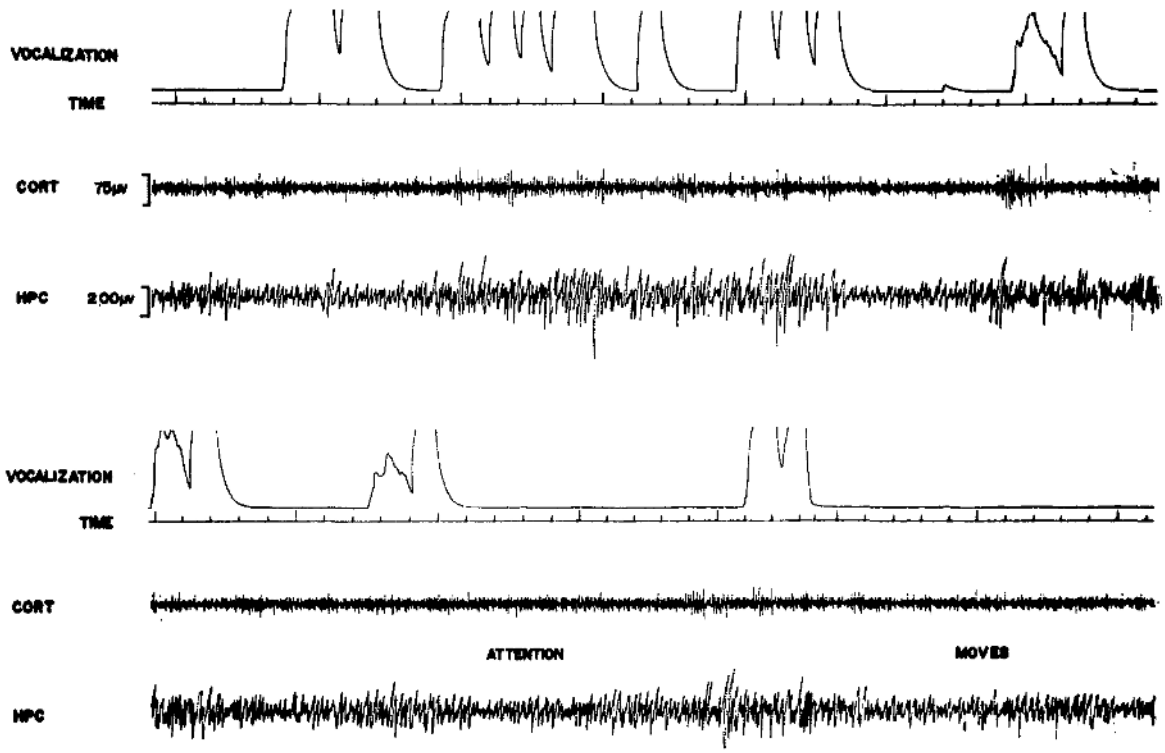

FIGURE 3. Cortical (CORT) and hippocampal (HPC) electroencephalograms during a period of transition from the vocalization period to the alert state of the postvocalization period. (Vacillation between a sleeplike and an alert state is demonstrated. Vocalizations appear sporadically in synchrony with the sleeplike state. Vocalization traces represent pulses of sound. Traces are on/off only; where tops are clipped off, pulse sounds continued. Dips between pulses represent intervals between vocalizations. When vocalization trace is flat, no sounds were emitted. Time marker is in seconds. Overt behavior is indicated.)

assure that there had not simply been a pause in the calling, but rather that the male had terminated. Considering this, the shock-to-intromission latency is important; this was 15 sec. or less in the 3 PEIs.

It was also observed that the preshock–wires-attached condition alone had an effect on the refractory period. The VT was significantly longer in the shock and preshock conditions than in the ad lib ($p < .025$). Al-

TABLE 2

CHARACTERISTICS OF THE HIPPOCAMPAL ELECTROENCEPHALOGRAM DURING DIFFERENT PHASES OF THE POSTEJACULATORY REFRACTORY PERIOD

Ejaculatory series	Vocalization latency period			Vocalization period[a]			Vocalization termination to PEI		
	% A	% SWS	Total time (in sec.)	% A	% SWS	Total time (in sec.)	% A	% SWS	Total time (in sec.)
1	89	11	215	11	89	620	93	7	373
2	89	11	280	16	84	432	90	10	425
3	84	16	388	5	95	345	96	4	257
4 and 5	92	8	262	6	94	1218	86	16	344

Note. PEI = postejaculatory interval; A = alert or aroused EEG, low voltage fast activity or theta rhythm; SWS = slow-wave, sleeplike EEG; Total time is the amount of time sampled from each of the periods.

[a] Records were analyzed for those portions where it was clear that the male was vocalizing. Where, during the vocalization period the male stopped vocalizing or the record was unclear, no analysis was carried out.

TABLE 3

EFFECTS OF SHOCK AND STIMULUS FEMALE ON MEASURES OF COPULATORY BEHAVIOR AND THE POSTEJACULATORY VOCALIZATION

Behavioral measure and ejaculatory series	Treatment group			F (df = 2/14)[a]	
	Shock	Ad lib control	Stimulus female	Series	Treatment
M Intromission frequency (IF)					
1	7.4	9.7	8.6	79.8*****	1.3
2	4.7	4.4	4.4		
3	4.6	5.5	5.0		
M Ejaculatory latency (EL)					
1	276	389	418		
2	180	181	172	17.2*****	6.2***
3	178	193	243		
M Vocalization latency (VL)					
1	35	54	34		
2	21	36	39	2.3	4.7**
3	25	52	54		
M Vocalization termination (VT)					
1	314	270	288		
2	365	329	320	23*****	3.1*
3	429	339	400		
M Postejaculatory interval (PEI)					
1	345	377	388		
2	422	441	475	78.1*****	8.4****
3	515	519	624		
M PEI − VT					
1	30	106	100		
2	57	111	154	11.6****	13.0*****
3	82	177	223		
M Ratio: VT/PEI					
1	.92	.72	.74		
2	.86	.75	.67	4.6**	15.6*****
3	.82	.65	.65		

[a] Data obtained by 2-way analysis of variance.
* $p < .10$.
** $p < .05$.
*** $p < .025$.
**** $p < .005$.
***** $p < .001$.

though PEI in the preshock condition was longer than in the ad-lib or shock condition, the difference was not significant.

DISCUSSION

The proportion of the PEI occupied by the postejaculatory vocalization was almost precisely the same in Experiment 1 as that presented in our initial report (Barfield & Geyer, 1972). We have found, too, that the perinatally androgenized female vocalizes following exhibition of the ejaculatory reflex for the same proportion of time, i.e., .74 ± .06 of the first PEI (Sachs, Pollak, Krieger, & Barfield, 1973). The male is generally immobile during the vocalization phase and *never* reinitiates copulation until after the cessation of this period. The reliability of this phenomenon and its constancy in independent sets of observations strongly support the contention that the vocalization period is the absolute refractory period first proposed by Beach and Holz-Tucker (1949).

It has recently been shown that the first two thirds of the postejaculatory refractory period is characterized by a predominance of slow-wave, high-amplitude EEG activity recorded from the hippocampus, and of cortical spindling (Kurtz & Adler, 1973).

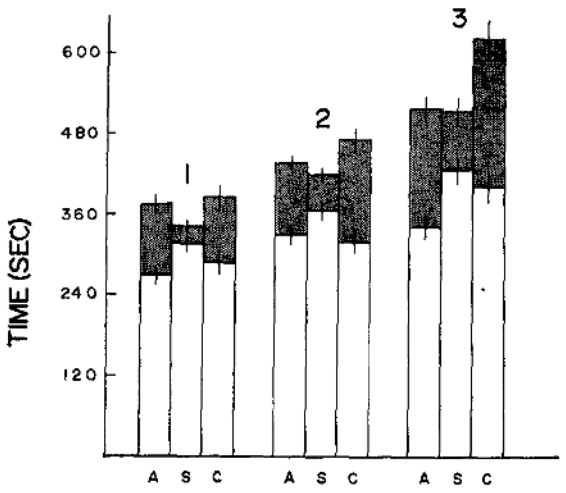

FIGURE 4. Effects of electrical shock on the postejaculatory vocalization (lower portion of bar) and postejaculatory interval (total bar). (Shaded portion = postejaculatory interval [PEI] — vocalization termination. Upper SE_M bars refer to the total PEI, the lower bars to the duration of the vocalization period. Ejaculatory series number is above each set of 3 bars. Abbreviations: S = shock condition; A = ad lib; C = changed stimulus female. Statistical information is presented in Table 3.)

This EEG pattern is characteristic of states of behavioral inhibition including sleep (Klemm, 1969). We have demonstrated here that the vocalization period is that during which the slow-wave EEG pattern predominates and, further, that the 22-kHz. vocalization is rarely observed without the presence of this sleeplike EEG pattern. Following the vocalization period, aroused EEG patterns predominate. Taken together, these observations substantiate the hypothesis that the end of the vocalizations signals the termination of the absolute refractory period and the beginning of the relative refractory period.

Immediately upon ejaculation, the male grooms the penis, and sometimes other activities occur, but vocalizations begin only after a short (35–50 sec.) latency period. This would suggest that the absolute refractory period does not begin until some time following ejaculation. There may, however, be 2 components to the absolute phase, spinal and cerebral. A spinal refractoriness of copulatory reflexes has been demonstrated by Hart (1968), but although it probably starts with ejaculation, its time course is not sufficiently long to account for the entire PEI or its absolute phase. It is quite possible that a cerebral component, characterized by a more generalized refractoriness, sets in only after some delay, but before the spinal phase dissipates.

The absolute refractory period may be severely curtailed under certain experimental conditions. In some cases, brain stimulation will cause the reinitiation of copulatory activity within a minute or less after ejaculation (Caggiula, 1970; Malsbury, 1971; Van Dis & Larsson, 1971; Vaughan & Fisher, 1962). Heimer and Larsson (1964) reported that rats with lesions in the area of the diencephalic–midbrain junction showed extreme reduction (to 1–3 min.) in the length of the PEI in 7 out of 9 animals. Perhaps electrical stimulation can at times override the cerebral component of the absolute refractory period, and likewise, lesions in the diencephalic–midbrain junction might destroy essential tissue for the production of this inhibitory state.

Larsson (1956) showed that following ejaculation the execution of a conditioned response (CR) was delayed 2½–4 min. on the average, in contrast to a delay of only 18–36 sec. following a majority of intromissions and presumably to no delay when sexual activity was absent. Although Larsson concluded that the PEI and the latency to CR after ejaculation were not under the control of the same mechanism, a consideration of the absolute refractory period might lead one to a modification of that position. The latencies to the CR were, on the average, and with increased experience, shorter than the PEI, at times as short as a minute or less. But usually they were of the same order of magnitude as the absolute refractory period relative to the PEI. Rats are not always quiescent, with vocalization and sleeplike EEG, after ejaculation. If alert during the absolute refractory period, they are not responsive to sexual stimuli, but might be attentive to CR cues. Larsson observed that mean CR latency after ejaculation increased with successive ejaculations; the absolute refractory period also increased with successive ejaculations. It would appear, then, that

TABLE 4

EFFECT OF PRETEST SHOCK AND SHOCK AFTER VOCALIZATION TERMINATION UPON COPULATORY BEHAVIOR AND THE POSTEJACULATORY VOCALIZATION

Behavioral measure and ejaculatory series	Treatment Group			F (df = 2/14)[a]	
	Preshock and postshock	Preshock only	Ad lib control	Series	Treatment
M Intromission frequency (IF)					
1	8.5	8.5	10.7		
2	4.3	5.0	5.0	27.1***	1.5
3	5.3	5.3	6.3		
M Ejaculatory latency (EL)					
1	519	768	663		
2	184	279	282	15.3**	.4
3	267	282	283		
M Vocalization latency (VL)					
1	30	34	47		
2	49	52	31	1.0	.5
3	37	55	45		
M Vocalization termination (VT)					
1	325	334	285		
2	391	399	333	40.4***	4.9*
3	475	438	372		
M Postejaculatory interval (PEI)					
1	361	457	376		
2	414	508	465	46.2***	1.5
3	508	540	574		
M PEI − VT					
1	36	121	91		
2	22	109	130	2.5	5.1*
3	33	100	208		
M Ratio VT/PEI					
1	.90	.75	.75		
2	.93	.79	.71	.1	5.5*
3	.93	.80	.65		

[a] Data obtained by 2-way analysis of variance.
* $p < .025$.
** $p < .001$.
*** $p < .0005$.

the inhibitory state responsible for the absolute refractory period is also responsible for the decreased responsiveness of males to a conditioned stimulus following ejaculation; however, in the latter case the control is not absolute.

It was proposed earlier that if the vocalization period truly was an indicator of the absolute refractory period, then shock would reduce the PEI in the shock condition to a value equal to the length of the vocalization period in the ad-lib condition. This had been suggested by the striking concordance between the reduction in PEI by shock originally reported by Barfield and Sachs (1968) and the proportion of the PEI occupied by vocalization (Barfield & Geyer, 1972). This was not precisely the case in Experiment 3; rather, the vocalization period tended to be lengthened by the application of shock. This unexpected finding will be discussed later, but it is significant here that once vocalization did terminate, mating was initiated about 30 sec. later as compared with about 100 sec. in the ad-lib condition. If the period from the termination of vocalization to the reinitiation of mating is indeed the relative refractory period proposed by Beach and Holz-Tucker (1949), then this period would be expected to be labile and broadly influenced by transient internal and external affects. When, in Experiment 4, shock was applied only after *spontaneous* cessation of vocalization, copulation was reinitiated

within 6–15 sec. of the shock. Clearly the male is capable of copulating as soon as he ceases to vocalize, and this confirms the hypothesis that the period following the termination of vocalization is the relative refractory period.

The absolute refractory period is quite stable, whereas the relative period is much more variable. Reference to Table 1 illustrates this point. The variance relative to the mean is much greater for PEI − VT than for VT. This difference suggests that different processes or sets of processes govern the absolute and relative periods.

Whereas the absolute refractory period is regulated by inhibitory processes, the relative refractory period is considered to be primarily under the control of arousal processes (Sachs & Barfield, 1974). Consistent with this view, the relative refractory period (i.e., PEI − VT) was characterized by a predominance of aroused EEG patterns.

Vocalizations of 22 kHz. are produced in 3 behavioral situations: (*a*) during aggressive behavior by subordinated or "badgered" rats (Sewell, 1967; Sales, 1972), (*b*) after electrical shock or other strong and probably stressful stimuli, and (*c*) during the absolute phase of the postejaculatory refractory period. When these vocalizations are emitted in any of these conditions, it is common for the animal to be in a state of behavioral immobility (freezing), and it follows that the 22-kHz. sounds may signal states of behavioral inhibition. If the inhibitory state is common, then it would be expected that the inhibition resulting from one condition (e.g., electrical shock) would be additive with that resulting from ejaculation. In Experiments 3 and 4, this was precisely the case, and one might conclude that a common inhibitory state can be brought into action by diverse behavioral antecedents.

The inhibitory mechanism that governs the absolute refractory period in some way promotes the occurrence of the 22-kHz. vocalization, and similarly, this inhibitory mechanism usually causes the animal to become quiescent and to demonstrate a sleeplike EEG. However, it is clear from the analysis of EEG records that there is not a strict correspondence between the absolute refractory period and the presence of either the vocalization or the sleeplike EEG pattern. In this connection, 22-kHz. vocalizations that follow defeat are at times accompanied by sleeplike EEG patterns and immobility, but a male displaying this behavior is capable of instantly initiating copulatory behavior (unpublished observations, 1973).

The postejaculatory vocalization is an integral part of the total copulatory behavior of the male rat. It would be indeed surprising if it did not play some important role in the reproductive biology of the species. At present we cannot state what that role might be; however, there are some possibilities that deserve further investigation.

The vocalization appears to keep the female away from the male, but not to cause her to escape. Thus, there is maintenance of separation, but without a loss of contact. From this, one might conclude that the vocalization functions to keep the female away from the male while he is incapable of copulatory response, but to maintain communication so that when vocalizations cease the female is signaled to reinitiate her sexual behavior.

One might, however, look deeper. The coordination of male and female reproductive physiology has been increasingly elucidated in recent years. The relationship between the multiple-intromission pattern of the male and the reproductive responses of the female has been well documented (Adler, 1969; Chester & Zucker, 1970; Edmonds, Zoloth, & Adler, 1972). It is quite possible that the ultrasonic vocalizations of the male rat effect the neuroendocrine responses of the female and in this fashion could be a significant reproductive influence. Sounds are significant in the reproductive response of birds (Barfield, 1971; Lehrman & Friedman, 1969); however, in rats only the deleterious effect of sound has been demonstrated. Of course, the sounds employed were in no way related to the naturally occurring sounds associated with the copulatory behavior of rats (Zondek & Tamari, 1967). Perhaps the postejaculatory vocalization is a sound that has a facilitative effect on reproduction; perhaps it is one more factor in the intricate interaction between the mating pair that ensures the fecundity of the species.

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