Pharmacology Biochemistry and Behavior, Vol. 41, pp. 551–558, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Morphine Attenuates Antipredator Ultrasonic Vocalizations in Mixed-Sex Rat Colonies

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Received 14 October 1991

SHEPHERD, J. K., D. C. BLANCHARD, S. M. WEISS, R. J. RODGERS AND R. J. BLANCHARD. *Morphine attenuates antipredator ultrasonic vocalizations in mixed-sex rat colonies.* PHARMACOL BIOCHEM BEHAV **41**(3) 551–558, 1992.—Mixed-sex groups of laboratory rats living in a visible burrow system (VBS) emit 18–27 kHz ultrasound and retreat to the burrow when a cat is placed in the open area of the VBS. The total duration of ultrasonic vocalizations was reliably reduced by pretreatment with 5 mg/kg morphine. In a subsequent study using male–female colony pairs, presentation of a cat to individual rats in the absence of their colony mate indicated significant gender differences in base frequency, degree of emission, and characteristics of pulses elicited. Specifically, females showed a greater number and duration of vocalizations, of higher frequency (kHz), and with shorter individual pulse durations than males. In the same study, morphine (5 mg/kg) produced a general decrease in the level of ultrasonic emissions in both sexes, reduced the mean base frequency (kHz), and increased the mean duration of individual pulses. These data suggest that endogenous opioid mechanisms may be involved in the mediation of ultrasonic vocalization in response to a predator, and are discussed with reference to known involvement of such systems in defensive responding.

Ultrasonic vocalization Alarm cries Morphine Anxiety Fear Antipredator defense
Sex differences Rat

ULTRASONIC vocalizations have been elicited in rodents in a wide range of situations and contexts including pup separation (23,34), sexual behavior (2), defense (26,42), pain (29), brain stimulation (51), startle (27), opiate withdrawal [see (32)] and predator exposure (9,10,12). While ultrasound research has focused to a large extent on vocalizations of young birds and mammals in response to separation from conspecifics (23,25,33,35), recent studies have returned to the phenomenon of adult ultrasonic vocalizations in the context of defense.

During conspecific agonistic encounters, intruder/defeated male rats were found to emit "short-pulse" ultrasounds of about 50 kHz and "long-pulse" ultrasounds of about 25 kHz frequency (40,42,47). The lower-frequency ultrasounds (25 kHz) have also been demonstrated in situations associated with pain (48) and in rats exposed to a natural predator, the domestic cat, a model that does not involve contact or painful stimulation (9,10,12). The antipredator vocalizations are similar in frequency (kHz), pulse form, and temporal characteristics to the 20 to 25-kHz cries produced by male rats in situations involving auditory startle stimuli (27) and, interestingly, differ from the postdefeat cries of male and female rats due to the absence of the higher frequency (30–70 kHz) component (31). The putative role of such vocalizations in conspecific communication can be illustrated by the somewhat specific circumstances required for ultrasound production with cat exposure. Thus, while substantial vocalizations are elicited when a cat is presented to a mixed-sex rat group living in burrow system colonies with tunnels and chambers, this is not the case with individual cat-exposed animals in an open area or for individual females in a novel apparatus with possibility of concealment, even with a repeat exposure 24 h later (10). These findings suggest the importance of an established group of conspecifics, together with perceived safety, both of which are consonant with the view that ultrasonic vocalizations may serve as alarm cries in the presence of a predator. Indeed,

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some evidence for this notion is provided by a recent report that "playback" of 22-kHz cries, recorded from defensive rats during conspecific agonistic interactions, markedly reduced locomotor activity and sniffing of the loudspeaker in individual rats in a neutral arena (41).

Recent analysis of antipredator defensive behavior has provided substantial evidence for pronounced gender differences. Thus, females show significantly higher levels of a variety of defensive responses to cat presentation, including freezing, avoidance of contextual stimuli paired with the cat, and active investigation of predatory odors (5). In the present context, a recent study in this laboratory demonstrated that females emit a greater number and duration of ultrasonic vocalizations than males in response to a predator (9), consistent with numerous other findings that revealed a higher level of female antipredator alarm vocalizations in several ground-dwelling sciurid species (22,44,46). In addition to a quantitative differentiation, detailed sonographic analyses revealed that females produced shorter individual pulse durations with higher base frequencies (kHz) and the negatively accelerated descending frequency pulse, characteristic of male cries, was not so common in the female response to predator exposure (9). While the reason for such differences remains to be elucidated, possible advantages in differential female vs. male alarm vocalizations have been proposed from a game theory perspective due to the longer association by the female with the offspring (46).

It has been suggested that rodent ultrasound may provide a useful tool in the study of the neurobiology of anxiety and the data from rat pup isolation studies indicating a bidirectional response profile with anxiolytic/anxiogenic compounds would certainly seem to support this view [for review, see (24)]. While opiates are not considered effective anxiolytics, it has been known for some time that exogenously administered opiates are potent modulators of audible "distress" vocalizations in puppies, chickens, guinea pigs, and dogs (35,36). Similarly, morphine (7.5 mg/kg) selectively reduced audible vocalizations to dorsal contact, vibrissae stimulation, and vocalization to an anesthetized conspecific without markedly influencing a wide variety of other behaviors considered reliable indices of fear/anxiety (7). In the present context, a range of μ-receptor agonists have been shown to decrease rat pup ultrasonic vocalizations [e.g., (15–17,50)], and recently morphine (3–10 mg/kg) has also been found to potently suppress both low- (20–29 kHz) and high-(30–70 kHz) frequency ultrasonic cries emitted by defensive adult male and female rats during agonistic interactions (32,49). Morphine (3–6 mg/kg) has also been found to decrease the total duration of 20- to 30-kHz vocalizations during the 5-min interval preceding noxious electrical tail stimulation. Interestingly, in the latter study morphine was found to change the nature of the ultrasound emissions from a characteristic bout structure to single pulses (48).

The intention of the present study is to assess the effects of morphine on rat ultrasonic vocalizations produced by cat exposure. The inclusion of a no-cat control situation is not necessary in this context as previous observations of a time-dependent return to baseline levels of ultrasound clearly indicate that these ultrasonic cries are closely associated with stimulus presentation (9,10,12). This investigation will involve analysis of responses by whole colonies and by individual males and females in the absence of their colony mates. The latter procedure is intended to confirm previous gender differences in ultrasound characteristics in addition to monitoring any sex differences in the effects of morphine.

EXPERIMENT 1

METHOD

Subjects

Eight colonies, comprising one male and two female Long-Evans rats (136–236 days old), were established in visible burrow systems (VBS's).

Drugs

Morphine sulphate (0, 1, and 5 mg/kg) dissolved in physiological (0.9%) saline, which alone served as control, was administered intraperitoneally in a volume of 1 ml/kg 30 min prior to behavioral testing.

Apparatus

The VBS consisted of two chambers (18 cm height) connected to an open surface area (85 × 60 cm) with no ceiling by tunnels constructed from clear Plexiglas tubing (7 cm diameter). A light baffle consisting of walls 90 cm in height surrounded the open surface area such that a 15-W fluorescent fixture located 45 cm above the surface area had minimal impact on illumination of the tunnels and chambers. Each VBS was located in a laboratory room that was maintained under continuous red light. The surface area was illuminated by incandescent light on a 12 L:12 D cycle (lights on 4 a.m.). An Ultrasound Advice S-25 bat detector set to detect frequencies in the range of 18–27 kHz (mode 22 kHz) was used to detect ultrasonic vocalizations. The output of the bat detector was connected to a videorecorder to provide a record of ultrasonic vocalizations throughout the test period.

Procedure

After 1 week of undisturbed residence, all animals were removed from each VBS and administered morphine (5 or 10 mg/kg) or saline, with all three animals of a given colony receiving the same dose. On the following 2 days, each colony received the remaining two treatment conditions (counterbalanced over days 1–3 for treatment condition). Following drug/saline administration, subjects were replaced in their respective burrow systems for 30 min prior to the introduction of a stimulus cat, one of several females selected on the basis that they did not attack rats, for 15 min. Ultrasonic cries (i.e., output from the bat detector) were recorded during cat exposure and for 30 min afterward (analyzed as two 15-min time bins). All testing was carried out during the initial 4 h of the dark period.

Analysis

The durations of ultrasonic vocalization by each colony for each 15-min time period (cat, post1, post2) were analyzed by analysis of variance (ANOVA). Total durations were calculated on the basis of the number of subjects vocalizing at a given time, that is, a period of 15 s vocalizing by all three animals provides a total duration of 45 s.

RESULTS

Total Duration of Vocalizations

Figure 1 shows the effects of morphine on the total duration of 22-kHz vocalizations emitted by colonies both during

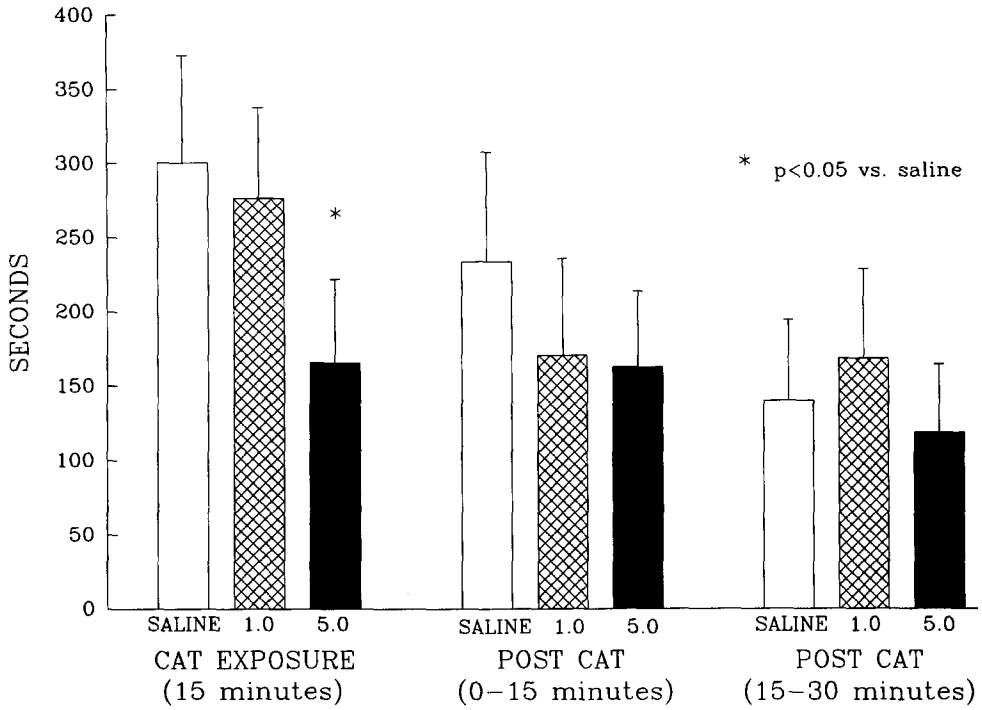

FIG. 1. Effects of morphine (mg/kg) on the total duration of ultrasonic cries emitted by colonies (one male, two females) during and after exposure to a cat. Data are mean durations (in seconds).

and after cat presentation. ANOVA for the 15-min cat period indicated a reliable effect of treatment condition on the total duration of ultrasonic vocalizations emitted, $F(2,22) = 3.50$, $p < 0.05$. The mean duration of vocalizations for saline-treated subjects was reliably greater ($p < 0.05$, t-test) than durations for animals receiving 5.0 mg/kg morphine and greater, although failing to reach statistical significance ($p < 0.07$), than the 1.0-mg/kg group.

ANOVA for the two 15-min postcat periods failed to indicate any reliable effects of morphine treatment on vocalizations. This absence of drug effects may be due to the decrease in control (saline) vocalizations from the cat period to the postcat periods (Fig. 1).

These findings clearly indicate a specific inhibition of antipredator ultrasonic vocalizations from colonies following morphine administration. However, these data do not provide any detailed information as to changes in frequency, modulation, and temporal parameters of such vocalizations, nor do they provide any insight as to gender differences in control responding or in the influence of morphine treatment. The following study was designed to elucidate these issues by investigating the effects of morphine on individual male and female subjects exposed to a cat in the absence of their colony mate. The Racal instrumentation allowed more detailed sonographic analysis of pulse form characteristics.

EXPERIMENT 2

METHOD

Subjects

Seven colonies, comprising one male and one female Long-Evans rat (130–288 days old), were established in VBS's.

Drugs

Morphine sulphate (0, 5 mg/kg) dissolved in physiological (0.9%) saline, which alone served as control, was administered intraperitoneally in a volume of 1 ml/kg 30 min prior to behavioral testing.

Apparatus

The apparatus was identical to that used in Experiment 1. In addition, a Racal store 4DS instrumentation recorder connected to an Ultrasound Advice SM2 microphone and a SP2 preamplifier was used to record vocalization for detailed sonographic analysis using a Kay DSP1 5500 digital sonograph.

Procedure

After 1 week of undisturbed residence in the colonies, all animals (including the test subject's colony mate) were removed from the colony room except for the required test animal. This ensured identification of the source of any ultrasound recorded during the test period. Thirty minutes after injection of either morphine or saline, one of the stimulus cats employed in Experiment 1 was introduced to the open area of the VBS for 15 min. Each subject received both treatment conditions over two successive days (counterbalanced for gender and order of administration). During cat presentation, the output of the bat detector was videotaped for analysis. The recording speed of the Racal instrumentation recorder was set at 15 in/s to ensure that any vocalization between 1- to 100-kHz frequency would be recorded with adequate fidelity. Due to the large quantity of tape required for such high-speed recording, only a 5-min session of ultrasound was recorded by the Racal for each subject during cat presentation, begin-

ning with the first ultrasound indicated by the bat detector. All testing was carried out during the initial 4 h of the dark period.

Data Analysis

All data from the bat detector and the Racal instrumentation recorder were analyzed by ANOVA.

RESULTS

Total Number and Duration of Vocalizations

Table 1 shows the mean total number and duration of vocalizations emitted by individual males and females receiving morphine or saline during 15-min cat exposure. ANOVA indicated that over the test period the total number of vocalizations was reliably decreased by 5 mg/kg morphine, $F(1,11) = 11.21$, $p < 0.01$ and that females emitted a greater number of vocalizations than males, $F(1,11) = 9.74$, $p < 0.01$. Similarly, ANOVA indicated that the total duration of vocalizations was reliably decreased by 5 mg/kg morphine, $F(1,11) = 14.26$, $p < 0.005$, and that total durations were greater for females than for males, $F(1,11) = 14.50$, $p < 0.005$. ANOVA also indicated a reliable sex × dose interaction, $F(1,11) = 10.36$, $p < 0.01$. Newman-Keuls comparisons confirmed a reliable reduction in durations of female, but not male, vocalizations by morphine ($p < 0.001$). This finding reflects the considerably lower control (saline) level of ultrasonic cries emitted by males vs. females ($p < 0.001$), suggesting a floor effect as the reason for the absence of morphine influence in the former.

Mean Base Frequency (kHz) of Vocalizations

Figure 2 shows the distribution of frequencies (kHz) emitted by males and females receiving morphine or saline; means are indicated in parentheses. ANOVA indicated that the mean base frequency (kHz) of vocalization was reliably decreased by morphine, $F(1,4) = 16.76$, $p < 0.02$, and that emitted frequencies were higher for females than males, $F(1,4) = 20.44$, $p < 0.02$.

Interpulse Intervals and Pulse Train Parameters

The sonographic records indicated that pulses of continuous vocalization were separated by interpulse intervals of widely varying length, suggesting an organization of pulses into "pulse trains" separated by longer intervals.

To describe characteristics of the pulse trains and intertrain intervals, we attempted to determine the maximum duration of an interpulse interval that should be regarded as separating pulses within a train such that longer-duration intervals could be defined as separating pulse trains. As described previously (9), distributions of interval lengths were plotted as survivor functions on a logarithmic scale, applying the technique of Machlis (30) and Bressers et al. (15). These distributions, comprising all interpulse intervals less than 7 s in duration, are presented in Fig. 3.

It has been demonstrated that randomly distributed interpulse intervals will produce an exponential distribution in the untransformed survivor plots or a linear function characterizing log survivor plots (21,30). The Kolmogorov-Smirnov goodness of fit test was applied to the survival functions for each subject and the results allowed rejection of the hypothesis that the individual distributions could be described by an exponential function for all subjects except male 4 (morphine) and male 1 (morphine). As Fig. 3 shows, these are the only two subjects that provide any evidence for a clear linear function, indicating a nonnormal distribution of intervals for all other subjects (i.e., interpulse vs. intertrain). Our criterion for separation of pulse trains was based on the interpulse interval data for each animal: Distribution of interpulse intervals from least to most within the 5-min period indicated that all animals had many interpulse intervals ranging in duration from about 70 ms to about 250–500 ms and closely grouped within that range. Individual records were examined for the first gap of 50 ms or more between adjacent interpulse intervals arranged in ascending order. A value 50 ms above the lower figure in that gap was then used as the minimum interval between pulsetrains for that particular subject. This break point is indicated for each subject in Fig. 3, which shows that these break points correspond very well to the inflection points of the distributions. Application of this individualized criterion produced individual values for separation of pulse trains that, in turn, were used to assess any influence of gender and morphine treatment on pulse train characteristics. Table 2 shows the mean pulse duration, interpulse interval, pulse train duration, intertrain interval, and number of pulses per train for individual males and females receiving morphine or saline.

ANOVA did indicate a reliable effect of gender on intertrain intervals, $F(1,4) = 86.25$, $p < 0.001$, with males showing longer interval durations than females. However, there were no reliable main effects for gender in terms of pulse durations, interpulse intervals, pulse train durations, or the number of pulses in a pulse train.

Similarly, ANOVA indicated that morphine treatment had a reliable effect on pulse durations, $F(1,4) = 11.07$, $p < 0.05$, without reliably influencing any other measure, although the main effect for dose did approach statistical significance for interpulse interval durations, $F(1,4) = 6.35$, $p < 0.06$. The former reliable effect was due to an increase in pulse durations with 5 mg/kg morphine, while the latter finding reflects a (nonsignificant) trend toward increased interpulse intervals with drug treatment.

TABLE 1

TOTAL NUMBER AND DURATION OF ULTRASONIC VOCALIZATIONS EMITTED BY INDIVIDUAL MALES AND FEMALES RECEIVING SALINE OR MORPHINE

	Saline	Morphine
Number of vocalizations		
Males	34.43 (24.59)	7.57 (7.24)
Females	502.5 (152.49)	79.29 (58.34)
Duration (in seconds) of vocalizations		
Males	30.39 (19.56)	8.57 (8.31)
Females	343.86 (83.98)	60.21 (36.12)

DISCUSSION

Present findings indicate substantial gender differences in ultrasonic responding during exposure to a noncontacting predator. Thus, females emitted a greater number and duration of vocalizations, with a higher base frequency (kHz) than males, confirming earlier studies in this laboratory (9,10,12). In the latter study, females were found to produce shorter individual pulse durations. While present data did not reliably

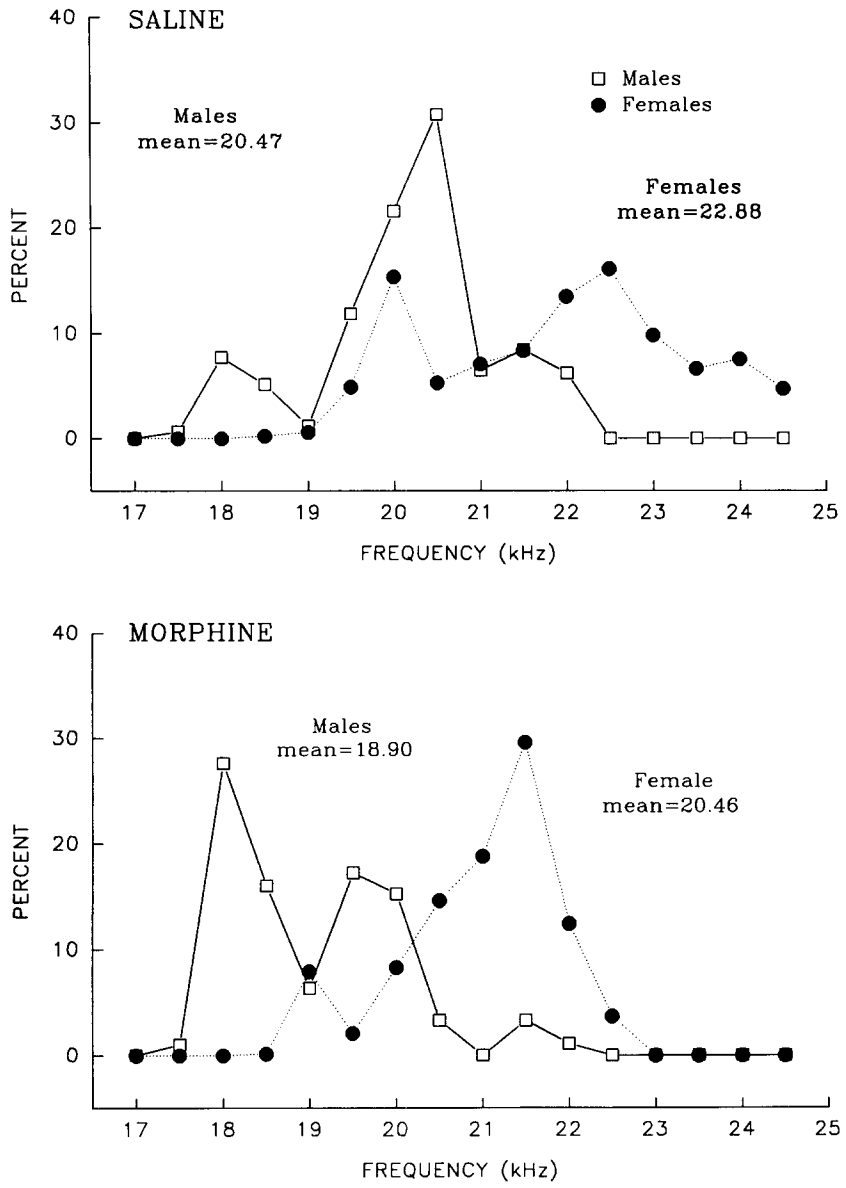

FIG. 2. Distribution of frequencies (kHz) emitted by individual male and female rats exposed to a cat following saline or morphine treatment.

confirm this phenomenon, perhaps due to the low number of males emitting ultrasound, there was evidence for a sexual differentiation in pulse form characteristics in terms of longer intervals between trains of pulses for males than females. Previous investigation has provided evidence for significantly higher levels of a variety of defensive responses to cat presentation by females than males. These include freezing, avoidance of contextual stimuli paired with the cat, and active investigation of predatory odors (5). This suggests that present gender differences may reflect a defensive function in predator-elicited ultrasonic vocalizations for colony-dwelling rats with an available place of concealment.

Morphine treatment produced a number of quantitative and qualitative changes in ultrasonic emissions. Thus, the total duration of vocalizations emitted by the colonies was reliably decreased by morphine during cat presentation, while the rapid dissipation of these emissions after removal of the cat made assessment of morphine effects somewhat arbitrary in the postcat period. Similarly, morphine reduced vocalizations by individual males and females, although the effect on males was less pronounced due to their lower control (saline) response levels. A qualitative influence of morphine treatment was indicated by the reduction in mean base frequency (kHz) and increased pulse durations. This overall reduction in ultrasonic responding is consistent with the well-documented effects of morphine on audible "distress" vocalizations (35,36), as well as the reduction of ultrasonic cries by opiate agonists in a wide variety of contexts (16–18,48–50).

Morphine has previously been found to produce a very selective inhibition of audible vocalizations to dorsal contact, vibrissae stimulation, and vocalization to an anesthetized conspecific without markedly influencing a wide variety of other

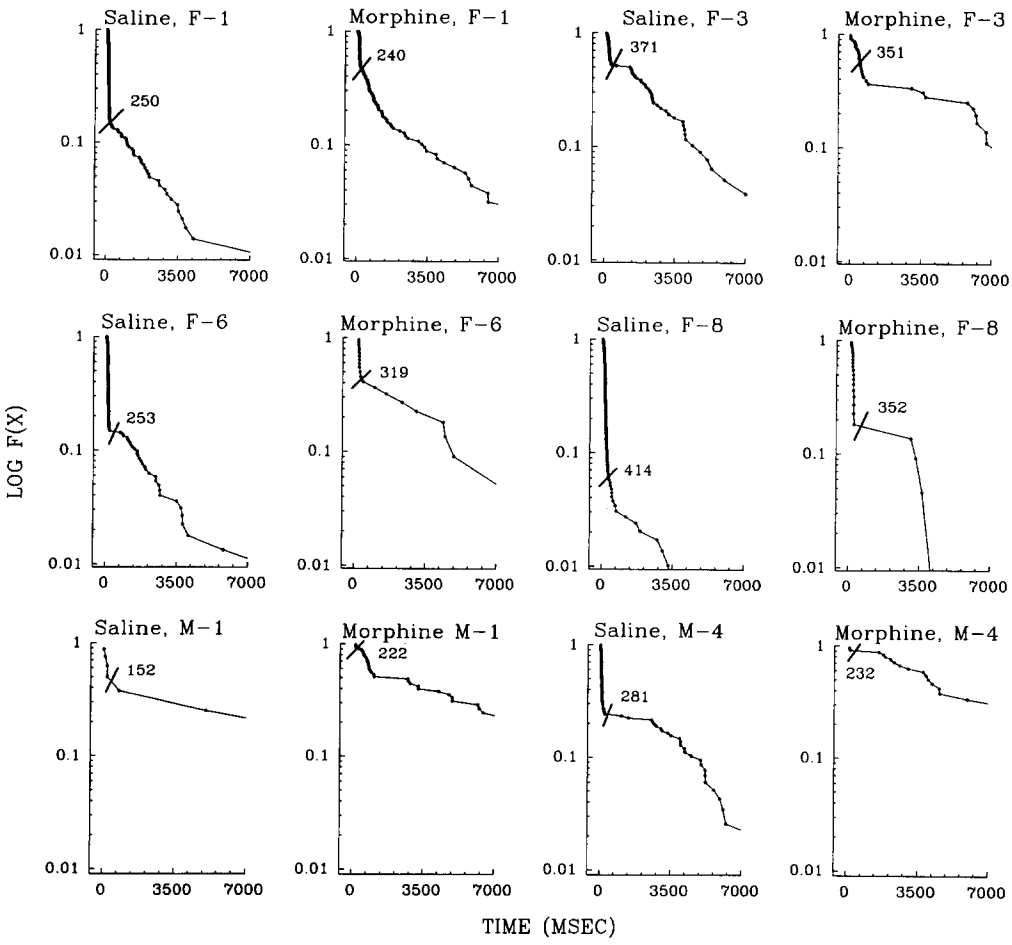

FIG. 3. Log survivor plots for interpulse intervals for individual male and female rats following saline or morphine treatment.

TABLE 2

MEAN PULSE AND PULSE TRAIN CHARACTERISTICS FOR INDIVIDUAL MALES AND FEMALES RECEIVING SALINE OR MORPHINE

	Saline	Morphine
Number of pulses/train		
Male	2.28 (0.83)	1.08 (0.01)
Female	5.57 (1.96)	2.52 (0.71)
Pulse duration		
Male	843.94 (260.33)	1372.21 (345.71)
Female	933.20 (113.70)	1064.01 (183.85)
Interpulse interval		
Male	99.62 (27.71)	165.83 (5.17)
Female	155.62 (29.97)	176.18 (28.51)
Pulse train duration		
Male	1987.96 (720.79)	1495.74 (386.44)
Female	5046.64 (1637.94)	2975.87 (1056.42)
Intertrain interval duration		
Male	5900.12 (836.13)	7864.21 (2236.66)
Female	3680.20 (695.18)	3229.95 (329.88)

defensive responses to full or partial predatory stimuli (7). Importantly, many of the latter defensive responses are attenuated by a number of traditional and novel anxiolytic compounds (3,4,6,8,11,43). Thus, while the view that opiates may prove useful in the treatment of anxiety disorders has long since lost its appeal and studies with morphine have not provided any reliable evidence for anxiety/fear reduction in terms of reduced antipredator behavioral responding (7), there does appear to be a highly specific interaction between the opioid system and ultrasonic vocalization, possibly representing a more subtle adaptive mechanism.

In this context, there is a well-established association between endogenous opioid release and antipredator defense (14,19). It would seem pertinent to suggest that if exogenous opiates serve to reduce ultrasound emissions then endogenous opioid release should correlate with those situations in which silence would be a more adaptive strategy than vocalization. Indeed, a recent study elegantly demonstrated a differentiation between such situations in terms of the ontogeny of adaptive responding in rat pups (45). This study reported that when 14-day-old pups are isolated in close proximity to an adult male, a potentially threatening stimulus, they emit significantly fewer ultrasonic cries that pups in close proximity to the mother or isolation per se. However, 7-day-old pups do not differentiate between situations. It is suggested that in

younger pups in the absence of effective ambulatory responses and physiological development (hypothalamic-pituitary-adrenal axis) a high level of ultrasound necessary for maternal retrieval is the only effective survival strategy, whatever the context. In contrast, the differential reactions of 14-day-old pups, far more capable of effective ambulation, probably reflect development of physiological systems or "hormonal support" for the rapid modulation of defensive behavioral responses in stressful situations (45). Admittedly, the above findings do not provide direct evidence for an association between endogenous opioid release and inhibition of ultrasound, but they do serve to illustrate the adaptive nature of such an inhibition in a defensive context. However, active opioid mechanisms have been demonstrated in 10-day-old (and subsequently 14-day-old) rat pups as the analgesia and the attenuation of ultrasonic vocalization, following milk infusion, were both reversed by naltrexone (13).

Clearly, such a differentiation between situations where silence or ultrasound is the most adaptive strategy would make sense in terms of our knowledge of the adult defensive repertoire. For example, if the prey animal were in a safe environment (e.g., close to the burrow entrance) upon perception of a predator, ultrasonic vocalizations, presumably functioning as conspecific communication or "alarm" cries, would not place the animal in any greater danger. However, if a predator-prey interaction were to reach the stage where tonic immobility becomes the only appropriate action, in which the prey apparently feigns death to promote loss of interest and increase the chance of escape from the predator (20,37), ultrasonic vocalization would not be adaptive. Importantly, an association between tonic immobility and concurrent activation of endogenous opioid systems has been demonstrated, albeit in the context of prolonged conspecific, rather than predatory, aggression (38,39). In addition, it has been demonstrated that body-pinch, believed to mimic handling by a predator, simultaneously induces tonic immobility and opioid analgesia in mice (1).

The final, and perhaps most convincing, argument for inhibition of ultrasonic vocalizations with concurrent activation of endogenous opioid systems comes indirectly from two separate studies. Thus, Lester and Fanselow (28) demonstrated that subjecting rats to 15 min of noncontact exposure to a cat resulted in a naltrexone-reversible analgesia. In an identical procedure using individually housed (nonsocial) rats with no available place of concealment, Blanchard and colleagues reported no evidence of ultrasonic vocalization (10,12). These findings clearly suggest an adaptive association between endogenous opioidergic activation and inhibition of ultrasound in the context of antipredator defense.

ACKNOWLEDGEMENT

This article was supported by NIH Grants MH42803 and RR03061.

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