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BRIEF COMMUNICATION

Diazepam Facilitates Stimulation-Induced Feeding in Rats

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BIELAJEW, C. AND T. BUSHNIK. *Diazepam facilitates stimulation-induced feeding in rats.* PHARMACOL BIOCHEM BEHAV **48**(2) 557–561, 1994. – The effect of diazepam on the trade-off function between current and frequency for stimulation-induced feeding was evaluated in five rats with electrodes implanted in medial forebrain bundle structures. Three of the five exhibited stimulation-induced feeding (SIF) in vehicle tests, while in the remaining two attention to food was interspersed with periods of high activity. In all cases diazepam facilitated stimulation-induced feeding; the expression of stimulation-induced feeding was observed at a dose of 2.5 mg/kg and 5.0 mg/kg, where tested, and frequency threshold shifts ranged from 10% to 25%. The degree of facilitation was consistent across currents in two of the four pairs of trade-off functions examined. The results suggest that diazepam can facilitate stimulation-induced feeding and its expression in feeding sites with a competing arousal component.

Stimulation-induced feeding Diazepam Medial forebrain bundle Current-frequency trade-off

IT has been known for some time that electrical stimulation of the lateral hypothalamus can produce a diversity of motivational effects, including the induction of feeding in food-satiated rats (5). Many of the characteristics of stimulation-induced feeding (SIF) appear to be similar to those of natural feeding, suggesting some overlap between the CNS mechanisms underlying SIF and those mediating normal hunger processes (39). For example, both forms of eating are affected by many of the same manipulations, including stomach loading (6), food deprivation (6,24,34), and adulteration (34), and to both, learned (40) and unlearned taste aversions (34) and, to some degree, classical conditioning have been attributed (18, 47). Likewise, there are parallels in the effects on SIF and natural feeding following the administration of many classes of pharmacological agents. Systemic administration of neuroleptics (15,19,28,33,41,43), the gastrointestinal peptide bombesin (11,13), and high doses of amphetamine and the anorexic compound fenfluramine (2–4,7,16,22,44) tend to suppress either spontaneous or deprivation-induced feeding and SIF, while the opiate morphine injected into the ventral tegmental area (20,21) peripherally administered Δ^9-tetrahydrocannabinol (17,35), the psychoactive component of marijuana and diazepam, a minor tranquilizer belonging to the benzodiazepine group of drugs (8,23,29–32,36,38,42) generally facilitate both forms of feeding.

It was the latter and its ability to facilitate SIF that was of interest in this study. Specifically, we exploited the anxiolytic property of diazepam to control the stimulation-induced arousal that appeared to be competing with the expression of SIF in some subjects, and in doing so we observed that diazepam had a significant effect on SIF frequency thresholds. Indeed, others have reported reduced current thresholds for SIF (31,38) and the demonstration of SIF following diazapam challenge in animals previously classified as noneaters (33). To further investigate this phenomenon, animals that exhibited SIF or signs of it from electrodes implanted in medial forebrain bundle structures were administered diazepam or its vehicle, and changes in the frequency thresholds associated with a wide range of currents were examined. The frequency scaling method was selected to insure that the population of stimulated neurons was held constant; current scaling is based on the assumption that the distribution of behaviorally relevant neurons is invariant as the size of the effective stimulation field is altered (9,11,14,45,46). To test whether threshold

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changes in response to diazepam are stable across currents, we evaluated the trade-off between current and frequency for SIF and the effects of diazepam on this function.

METHOD

Subjects and Surgery

Five male Long-Evans rats (Charles River Laboratories, St Constant, Quebec) weighing 340–440 g at surgery were individually housed in plastic cages and allowed free access to Purina rat chow and water. They were maintained on a 12-h light-dark cycle with light onset at 0700. Stereotaxic surgery was conducted under sodium pentobarbital anesthesia (65 mg/kg Somnotol, IP) and a SC injection of atropine sulphate to reduce respiratory distress. With the head oriented in a flat-skull position, each animal was implanted with a single monopolar stimulating electrode; the one exception, TH11, had a bilateral lateral hypothalamus (LH) assembly. The LH coordinates ranged from 2.0 to 3.0 mm posterior to bregma, 1.6 to 1.7 mm lateral to the midsagittal suture, and 8.2 to 8.4 mm below the skull surface reading at bregma. The ventral tegmental area (VTA) electrode was aimed posteriorly 4.3 mm, laterally 1.2 mm, and ventrally 8.8 mm. All coordinates were based on the Paxinos and Watson atlas (27).

Apparatus

The electrodes were fashioned from 250-μm-diameter stainless steel wire, insulated with Epoxylite to the flattened tips. A flexible stainless steel wire wrapped around four stainless steel skull screws served as the current return. The entire electrode assembly was anchored to the skull screws with dental acrylic.

Behavioral tests were conducted in a wood and Plexiglas box with dimensions 28 × 38 × 44 cm. The floor of the chamber was covered with rat chow pellets.

Electrical stimulation was supplied by constant-current amplifiers (26) and integrated-circuit pulse generators built in-house. The current was continuously monitored on an oscilloscope by reading the voltage drop across a 1-Ω precision resistor in series with the rat. Charge polarization at the electrode tip was prevented by shunting the current between pulses to a low resistance path to ground (26). The stimulation consisted of a 20-s train of rectangular, monophasic, cathodal pulses of 100-μs duration; the trains alternated between a 20-s-on and 20-s-off schedule. Pulse frequency and current were varied according to the dictates of the screening and testing protocols described below.

Drugs

Diazepam was obtained from Hoffman-LaRoche (Nutley, NJ) in injection ampules with a concentration of 5 mg/ml. The vehicle solution (pH = 7.3) was prepared from 414 mg propylene glycol and 80 mg ethanol per milliliter of saline and was filtered before each injection.

Procedure

Screening. The presence of SIF was evaluated at currents of 50 μA, 100 μA, and 150 μA with the inclusion of 200 μA and 250 μA if feeding was absent and signs of aversion and motoric disruption were not observed at the lower currents. Three pulse frequencies—20, 32, and 50 Hz—were tested at each current. For a positive rating, feeding had to be initiated during the 20 s of stimulation and terminated as soon as the 20-s-off phase began. Screening began with the lowest current (50 μA) and frequency (20 Hz) values, after which the frequency was increased to 32 Hz and 50 Hz for the remaining two trials at that current. The current was then increased by 50 μA and the ascending frequency series repeated. Each current-frequency combination was tested twice within a session; if SIF was not observed, the screening procedure was repeated a second day before the animal was classified as a nonfeeder. If SIF was observed, the current at which the behavior occurred was held constant and its associated frequency reduced in 0.05 common log unit steps until SIF was not elicited for two successive frequency values. The threshold corresponded to the first frequency at which the rat did not exhibit SIF.

Stabilization. When the frequency thresholds obtained at one current were relatively stable within a session, the trade-off between current and frequency was generated. Frequency thresholds were determined for about eight current values, ranging from 50 μA to 800 μA. To assess within-session stability, the frequency threshold of a midrange current (e.g., 200 μA) was determined at the beginning, middle, and end of each session, using a rule of no greater than a 0.10 log unit deviation during that session. Drug tests began when the thresholds associated with each current met this criterion.

Drug tests. Four of the five rats were evaluated with the vehicle and a 2.5-mg/kg IP dose of diazepam; the fifth, TH11, received in addition a dose of 5.0 mg/kg. Diazepam and its vehicle were administered on alternate days; within each testing session the currents were presented randomly. Tests began 10–15 min after injection to allow the overt signs of the sedation induced by diazepam to subside. Sessions lasted a maximum of 1 h, during which stable baseline measures were always observed.

One subject, URF62, represented a special case. The control trade-off function was generated before drug tests began and without the administration of the vehicle. Following this,

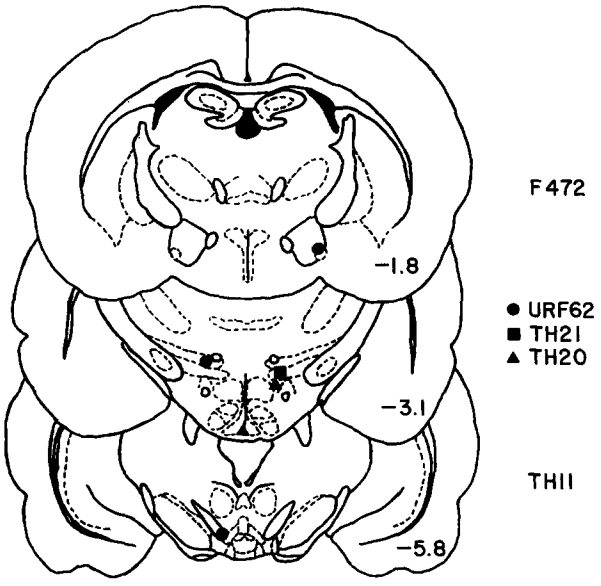

FIG. 1. Tracings from the Paxinos and Watson atlas plates that best match the sections containing the electrode tips. The corresponding subjects are identified on the right side of the figure; the anteroposterior coordinate is recorded on the bottom right side of each drawing. From Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates, 2nd ed. New York: Academic Press; 1986. Adapted by permission.

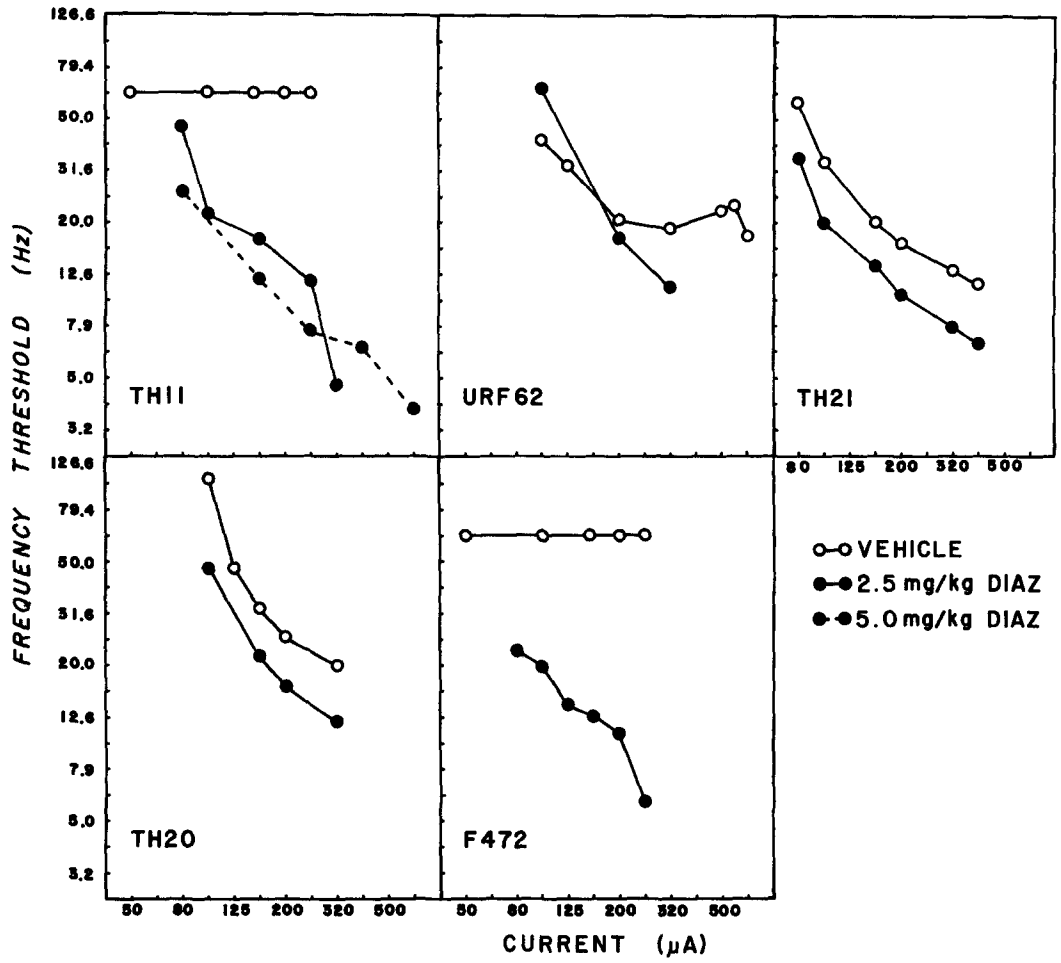

FIG. 2. The figure shows the current-frequency trade-off functions for each subject. The 5.0-mg/kg condition was tested only in TH11. The flat lines associated with TH11 and F472 indicate that in vehicle tests SIF was not observed at any of the frequency-current combinations.

the 2.5-mg/kg dose of diazepam was administered on alternate days until the stability criteria were met. This animal was included because negligible differences between "no-injection" and vehicle thresholds had been found in all other rats. For example, in TH20 at a current of 200 μA, the SIF frequency thresholds were 25 Hz and 26 Hz for the no-injection and vehicle conditions, respectively.

Histology. Animals were perfused intracardially with a solution of 0.9% saline followed by 10% formalin. The brains were removed and stored for at least 72 h in 10% formalin. They were sectioned at a thickness of 40 μm and stained with cresyl violet. The location of the electrode tips was based on the Paxinos and Watson atlas (27).

RESULTS

Histology

All of the electrode tips were located in the targeted structures (see Fig. 1).

Drug Tests

All five subjects exhibited a significant facilitation of SIF after diazepam challenge. Two, TH11 and F472, did not display SIF during the vehicle tests, but following the administration of diazepam, robust SIF was observed. The trade-off functions obtained from individual animals are shown in Figure 2. The data are ordered according to electrode location with the most posterior placement (TH11) in the upper left box and the most anterior site in the lower right box (F472).

Regression analyses were conducted on the frequency-current trade-off functions corresponding to the different drug conditions. The results of the statistical analyses, which include the regression F values and, when significant, the t values for the analyses of differences between the slopes and y-intercepts, are presented in Table 1.

For TH11, the regression analysis was applied to the threshold data collected during the 2.5- and 5.0-mg/kg conditions, since SIF was not observed in the vehicle tests; the resulting F value was significant, as were the slope and intercept differences. No statistical analysis was performed on the data from the second animal, F472, which did not exhibit SIF in vehicle tests and received a single dose of 2.5 mg/kg of diazepam. Of the remaining three subjects, all showed highly significant F values, suggesting that the diazepam and vehicle trade-off functions could be represented by separate regression lines; however, only in the case of URF62 were significant slope and y-intercept differences found.

TABLE 1
REGRESSION AND *t*-TEST ANALYSES OF FREQUENCY-CURRENT TRADE-OFF FUNCTIONS

	Overall *F* Regression	*t*-Test Slope	*t*-Test Intercept
TH11	3.883* (2,25)	2.073* (25)	2.798† (25)
URF62	13.764† (2,29)	5.099† (29)	8.709† (29)
TH21	42.194† (2,66)	0.163 (66)	1.002 (66)
TH20	22.596† (2,42)	0.354 (42)	1.467 (42)

Degrees of freedom are in parentheses. *$p < 0.05$. †$p < 0.01$.

Noteworthy were several behavioral differences that were observed between the vehicle and diazepam testing sessions. First, SIF tests in all subjects progressed more smoothly after diazepam was administered and the animals appeared less sensitive to extraneous noise. Second, in drug tests, SIF was exhibited over a wider range of currents and frequency thresholds were more stable; the error term, which was set at a maximum of 10% of the mean threshold value at any current, tended to be less in drug tests. Third, the drug improved the correspondence between the onset and offset of stimulation and the initiation and cessation of feeding.

DISCUSSION

In this study the frequency thresholds for SIF were clearly facilitated, with decreased values in three subjects and the induction of SIF in the remaining two. The results of the analyses in Table 1 suggest that the data share the following characteristics: First, the functions that relate the trade-off between current and frequency are best described as separate lines in all cases (i.e., between vehicle and drug) except in TH11, where the trade-off functions represent two doses of diazepam. Second, the vertical position, rather than the shape of the functions, appears to contribute more to this finding. Less consistent is the magnitude of this effect across subjects, with significant slopes and intercepts in two out of four cases. The observation of dissimilar slopes in TH11 and URF62 highlight a potential concern in studies of this nature and suggests that drug effects need to be evaluated at several currents. Overall, the pattern of results in these two cases is consistent with the other subjects. In TH11 the 5-mg/kg dose of diazepam was generally associated with lower frequency thresholds for SIF than was the 2.5-mg/kg one, and in URF62 diazepam produced lower thresholds more often than did the vehicle; a single test current, however, might have yielded a different conclusion. For instance, we have recently examined the effects of several doses of bombesin on SIF and found greater increases in frequency thresholds at low currents than at high ones (13). While this argument does not apply to all placements—note the pairs of trade-off functions obtained from TH20 and TH21 in which almost identical slopes were found—the use of current-frequency trade-off functions should reduce the risk of interpretation error.

Finally, the range of slope values obtained in vehicle tests (O.7–0.8) is consistent with Waraczynski and Kaplan's recent observations (37) and agree with our own large collection of SIF trade-off functions from the medial forebrain bundle (unpublished data); in comparison to self-stimulation, for example, where the relationship between current and frequency has been well-documented (9,10), SIF functions are profiled by shallower slopes and higher intercepts, suggesting a different integrative function from that which characterizes self-stimulation.

It has been reported that diazepam will induce SIF in animals that otherwise do not exhibit the behavior (31). We found in this study that the phenomenon is peculiar to placements that are feeding-related and does not reflect a nonspecific action of diazepam to induce SIF at any site. For example, bilateral LH electrodes were implanted in TH11, only one of which supported SIF after diazepam administration; we never observed feeding or any indication of its emergence in the contralateral site. In fact, in this laboratory we have never been able to induce SIF with diazepam in animals with clearly negative SIF sites. Thus, diazepam appears to be interacting with specific feeding-related mechanisms elicited by electrical stimulation (39). The demonstration by Watson et al. (38) that diazepam decreased current thresholds for SIF without affecting stimulation-induced drinking supports this notion.

Soper and Wise (31) have suggested that the anxiolytic property of diazepam induces SIF in noneaters by reducing the competing arousal produced by electrical stimulation. Our behavioral observations are consistent with this idea. In some of our subjects, the striking arousal caused by stimulation often appeared to be interfering with the expression of SIF in control tests. For example, URF62 would pick up the food at stimulation onset and then immediately drop it and run wildly around the cage until the offset period. Diazepam suppressed this behavior, and in less-agitated subjects improved the correspondence between the onset and offset of the stimulation and the initiation and cessation of SIF. A similar effect of diazepam has been observed in other stimulation-induced behaviors. Stimulation of the ventral inferior colliculus produces both switch-off or stimulation-escape and wild-running behaviors; dorsal inferior colliculus stimulation, however, does not elicit the switch-off response and the induced wild running outlasts the stimulation. Following diazepam administration, the poststimulus wild running seen in the dorsal site is eliminated, the switch-off response appears, and both behaviors are identical to those observed after ventral inferior colliculus stimulation (1). Likewise, Morgan et al. (25) have reported that in 60% of their periaqueductal gray placements diazepam reduced aversion reactions to periaqueductal gray stimulation and unmasked stimulation-induced analgesia. Although the expression of each of these stimulation-induced behaviors may result from the anxiolytic action of diazepam, the drug influence appears to be a specific one and does not act to generally disinhibit responding (38). In fact, in the case of stimulation-induced reward or self-stimulation, frequency thresholds are minimally affected by diazepam at placements that do not give rise to motoric seizures (12).

In this study we have shown that diazepam, similar to its effects on normal feeding, facilitates SIF and its expression in sites with a significant anxiogenic component when the relevant neuronal elements contribute to the effective stimulation field.

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