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High-Frequency Ultrasonic Vocalization Induced by Intracerebral Glutamate in Rats

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FU, X.-W. AND S. M. BRUDZYNSKI. *High-frequency ultrasonic vocalization induced by intracerebral glutamate in rats.* PHARMACOL BIOCHEM BEHAV **49**(4) 835–841, 1994.—Direct injection of glutamate, a neuroexcitatory agent, into the anterior hypothalamic-preoptic area of the rat brain induced ultrasonic vocalization. This vocalization was characterized by short-duration calls (below 60 ms) of high sound frequency (pitch), mostly above 40 kHz, and was similar to the known 50-kHz vocalization observed in natural situations. The glutamate-induced vocalization was dose dependent within the dose range of 16.9–67.6 μg and was antagonized by local pretreatment with MK-801, an NMDA antagonist. The increasing dosage of glutamate induced more calls and had a significant influence on frequency and intensity of emitted ultrasound. The average sound frequency increased whereas the mean sound intensity decreased with the dosage of glutamate. On the other hand, the mean duration of a single call and the bandwidth did not significantly change with doses of glutamate. Injection of carbachol, a muscarinic cholinomimetic agent, into the same brain sites as glutamate, induced a different type of ultrasonic vocalization with low sound frequency and long call duration, known as 22-kHz calls. The results suggest that high sound frequency, short-duration calls (50 kHz) and low sound frequency, long-duration calls (22 kHz) have different neurophysiological and neurochemical mechanisms.

Animal communication Ultrasonic vocalization 22-kHz calls 50-kHz calls Glutamate Carbachol
Anterior hypothalamic-preoptic area Rat

ULTRASONIC vocalization of rats has been recorded in a variety of behavioural situations and is thought to be associated with emotional and motivational states, particularly with dangerous and/or stressful situations (2–4,11,17,18,21,22). It has been documented that rats endangered by an offensive aggressor, by the presence of a predator, startled, exposed to painful stimuli, or even approached by man in an unfamiliar environment vocalized with 20–32 kHz, long calls known as the 22-kHz vocalization (3,6,12,13,16). Ultrasonic vocalization characterized by the same acoustic features may be induced pharmacologically by direct cholinergic stimulation of localized regions of the rat brain. Intracerebral injection of carbachol, an acetylcholine agonist, into the rat anterior hypothalamic-preoptic area reproducibly induced 20–32-kHz vocalization (5) that was comparable with ultrasonic vocalization induced by foot shock or by hand touch (11). Intracerebral carbachol induced a series of predominantly long calls (70% of calls longer than 300 ms) with narrow bandwidth (below 5 kHz) and constant frequency (5,11). Vocalization induced by carbachol also contained a component of short calls (40–300 ms) that were mostly retained within the same range, between 20–32 kHz [see sonogram in (11) and unpublished results]. Our recent study of the 22-kHz vocalization induced by hand touch revealed that it also contains two subpopulations: long calls (310–3940 ms) and short calls (20–300 ms) (8). However, this study has confirmed that, not only the long calls, but also 80% of short calls were maintained within the sound frequency (pitch) range from 20 to 32 kHz (8).

On the other hand, it is known that rats can emit short, high-frequency calls of 35–70 kHz that were observed during aggressive encounters, in response to an anesthetized rat, or after tail pinching (1,4,17,20). This short-call vocalization (below 65 ms), referred to as 50-kHz calls, represents a clearly distinct population of calls mostly observed in different behavioural situations rather than in those characterized by long 22-kHz calls (2,20). It has been suggested that, in natural conditions, the 22-kHz calls are associated with antipredator defensive behaviour whereas 50-kHz calls are associated with

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some other interactions among conspecifics (3,4). The short, high-frequency calls have not yet been induced by chemical stimulation of the brain and their neurochemical substrate is unknown. The present study has been undertaken to demonstrate and investigate pharmacologically evoked short, high sound frequency ultrasonic calls. The response will be induced by excitatory amino acid stimulation, which has been used to evoke other responses from the brain (9,15).

The goal of the study was 1) to characterize ultrasonic vocalization induced by injection of glutamate into the anterior hypothalamic-preoptic region in the rat brain; 2) to study the dose-response relationship and the type of glutamate receptor involved in the response; and 3) to compare ultrasonic vocalization induced by carbachol and glutamate from the same brain sites in the same animals. The results of the study will contribute to better understanding neurochemical mechanisms underlying production of short and long calls in rodents.

METHOD

Animals and Surgery

Twenty-five male Wistar rats (250–450 g of b.wt.) were used in the study. Animals were kept in single stainless steel cages with a 12 L : 12 D cycle and had standard pellet food and water ad lib. Rats were taken for experiments during their early hours of the light phase, 3–5 days after arrival in the animal quarters.

Animals were anaesthetized with ketamine hydrochloride (40 mg/kg, IP, Ketalean, MTC Pharmaceuticals, Cambridge, ON) followed by xylazine hydrochloride (3.2 mg/kg, IM, Rompun, Bayvet Div. Chemargo, Ltd., Etobicoke, ON); they were placed in a Kopf stereotaxic apparatus and bilaterally implanted with stainless steel guide cannulae (640 μm o.d.) into the anterior hypothalamic-preoptic area. The stereotaxic coordinates were: A = 8.7–7.7 mm from the interaural plane, L = 0.2–1.5 mm from the midline, and V = 8.0–9.0 mm below the surface of the cortex according to the stereotaxic atlas by Paxinos and Watson (19).

Intracerebral Injection Procedure

Sodium L-glutamate (monosodium salt of L-α-aminoglutaric acid, Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water and all other drugs were dissolved in sterile saline. The drugs were injected unilaterally into the brain by a Hamilton constant rate microsyringe (CR 700) connected with the stainless steel injecting cannula (310 μm o.d.) by a polyethylene PE-10 tubing (610 μm o.d., Clay Adams, Parsippany, NJ). L-Glutamate was injected into the brain in a volume of 0.2 μl of 0.5, 1, or 2 M solution [i.e., in doses of 16.9, 33.8, or 67.6 μg/0.2 μl (15)], and at a rate of 4 nl/s. All brain sites included in the investigation were injected with 1 M solution of glutamate. Injections of different doses of glutamate were given randomly 1 week apart in nine rats [for other details see (9)]. Carbachol (carbamylcholine chloride, Sigma Chemical Co.) was given in a dose of 1 μg/0.2 μl (5.5 mM solution) into the same brain sites as glutamate. Injections of 0.2 μl of vehicle served as control in all animals. Injections of glutamate and carbachol were counterbalanced in 12 animals (i.e., half of the animals received glutamate and half carbachol as their first injection followed by saline 1 week later). Other animals were injected in the same manner with glutamate (n = 20) and saline (n = 15) as their first injection. Then, all animals were injected with different doses of glutamate in random order in 1-week intervals. Responses induced by 33.8 μg of glutamate (1 M solution) in 10 rats were antagonized by a local pretreatment with 0.34 μg/0.2 μl (0.1 M solution) of (+)-MK-801 hydrogen maleate (dizocilpine maleate, Research Biochemicals Incorporated, Natick, MA), an NMDA antagonist (23). MK-801 was injected into the same brain site as glutamate 5 min before glutamate or was given alone 1 week later. To minimize number of injections/rat, animals that received MK-801 were not injected with all doses of glutamate.

Recording and Analysis of Vocalization

Animals were subjected to three adaptation tests during three consecutive days to avoid vocal responses to handling (6). Rats were observed and recorded during a 10-min session after injection and were not disturbed during that time. Immediately after injection, the animal was transferred into the observation cage. Vocalization was recorded in a padded, echo-free observation cage measuring 25 cm wide × 18 cm deep × 18 cm high. The cage was housed in a larger sound-resistant, ventilated, and temperature-controlled cubicle (BRS/LVE Tech Serv, Beltsville, MD). An ultrasonic microphone model SM1 (Ultra Sound Advice, London, England, working range 10–180 kHz) was mounted in the centre of the cage ceiling and was connected to the S200 bat detector (QMC Instruments Ltd, London, England). Presence of eventual vocalization during handling or injections was tested by a Mini-2 bat detector (Summit, Birmingham, England). Signals from the broadband output of the S200 bat detector with the frequency division ratio 1/16 were recorded on a tape recorder (VSC-2001, Tandy Corp.). Response latency expressed in seconds (from the termination of the injection to the first vocalization) and response duration expressed in minutes (from the first to the last vocalization after which the rat did not vocalize for at least 2 min) were also measured. Signals from the tapes were subsequently analyzed by a sonograph (DSP Sona-Graph signal analysis work station, model 5500, Kay Elemetrics Corp., Pine Brook, NJ) to obtain sonograms and power spectra from single calls. The results were printed on a Gray Scale printer (Model 5509, Kay Elemetrics Corp.). A detailed analysis of frequency, duration, bandwidth, and relative intensity was done manually using the sonograph cursors. First, the duration of individual calls was measured by reading the time span between two cursors positioned at the beginning and end of each call's sonogram. Then, the power spectrum peak was measured. For the selected input frequency and transform size, frequency and time resolution for sonograms were 20 Hz and 1.56 ms (for short calls below 300 ms), 20 Hz and 6.25 ms (for long calls above 300 ms), and 59 Hz and 25 ms for power spectra, respectively. The bandwidth was measured as the frequency difference between two cursors positioned at the beginning (the lowest frequency) and the end (the highest frequency) of the spectrogram peak. The relative power of calls was calculated by the sonograph from the waveform display logarithmically in 100-ms steps, referenced from the top of the scale and expressed in negative dBs.

Histological Verification and Statistics

After completion of experiments, animals were injected with 0.1 μl of 2 : 1 diluted suspension of India ink, sacrificed by an overdose of sodium pentobarbital, and perfused transcardially with 10% solution of formalin. The brains were fixed in 10% formalin, sectioned on a freezing microtome (Leitz, Wetzlar, Germany, model 1320) for 60-μm preparation, air dried, and stained with thionine. Histological preparations

were analyzed under microscope, and a map of injection sites was composed using a drawing attachment to the SZH zoom stereomicroscope (Olympus Optical Co., Japan). Marks of injection sites corresponded to the India ink depositions. Of 50 cannulae, 47 sites were injected with glutamate, and their localization is shown in Fig. 1. A vast majority of injection sites was located in or at the border of the anterior hypothalamic-preoptic area.

The numeral data were analyzed with Friedman's ANOVA for matched groups, Wiloxon signed rank test for matched pairs, Spearman's correlation test, and two-tailed *t*-test for correlated or independent comparisons, where applicable.

RESULTS

Localization of 47 injection sites is illustrated in Fig. 1. All the sites were injected with glutamate, which induced ultrasonic vocalization from 30 sites (circles in Fig. 1). Twelve sites, from which glutamate induced vocalization, were also injected with carbachol (asterisks within circles), which resulted in a different type of calls that were induced from all 12 sites.

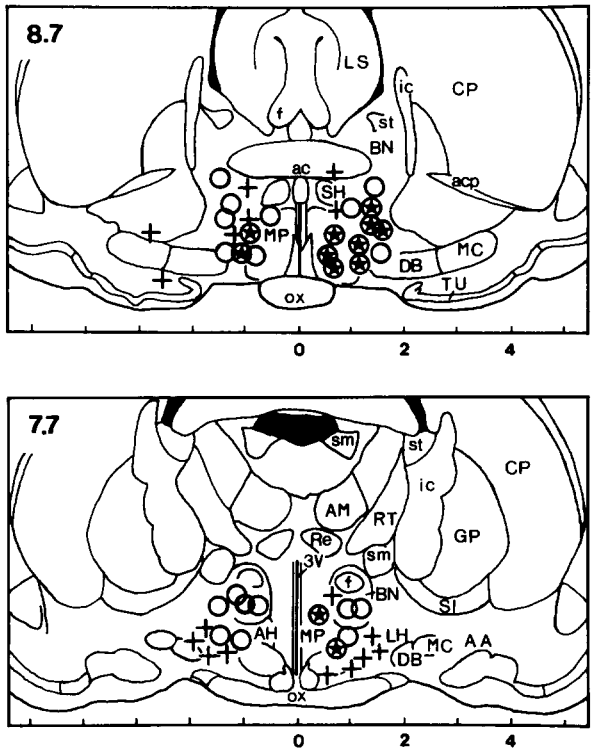

FIG. 1. Localization of 47 injection sites in the anterior hypothalamic-preoptic area and the neighbouring structures of the rat brain on the frontal planes A = 7.7 and 8.7. Glutamate (0.5-2.0 M solution) induced ultrasonic vocalization from 30 sites (circles). Ineffective sites are marked with crosses. Injection of carbachol (given mostly on the left side of the brain) into 12 out of 30 sites (stars) induced a different type of calls. Injection sites on the left and right side of the diagram correspond to the left and right side of the rat brain. Abbreviations: AA, anterior amygdaloid area; ac, anterior commissure; acp, posterolateral part of the anterior commissure; AH, anterior hypothalamic area; AM, anteromedial thalamic nucleus; BN, bed nucleus of stria terminalis; DB, diagonal band; CP, caudatoputamen; f, fornix; GP, globus pallidus; ic, internal capsule; LH, lateral hypothalamic area; LS, lateral septal nucleus; MC, magnocellular preoptic nucleus; MP, medial preoptic area; ox, optic chiasm; Re, reuniens nucleus; RT, reticular thalamic nucleus; SH, septohypothalamic nucleus; SI, substantia innominata; sm, stria medullaris; st, stria terminalis; TU, olfactory tubercle.

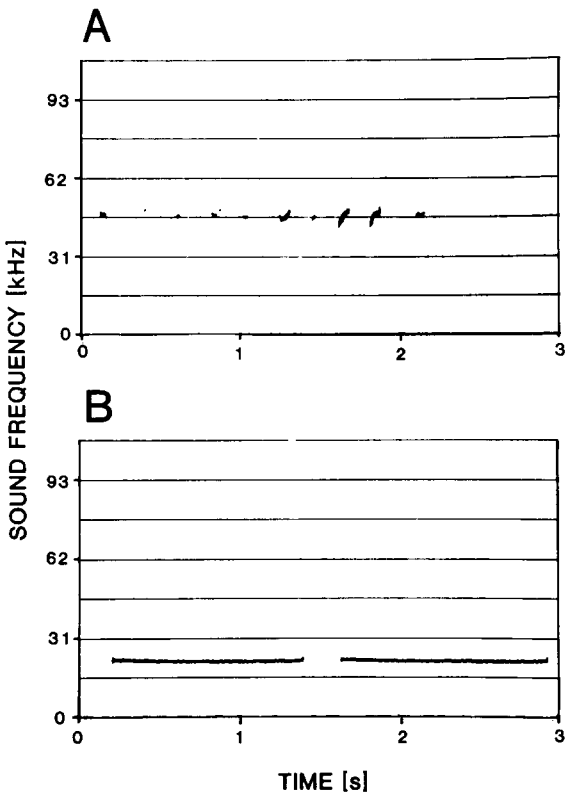

FIG. 2. Sonograms of ultrasonic calls induced by glutamate (A) and by carbachol (B) injected into the same brain site. Duration and frequency of the strongest call in (A) is 75 ms and 46.7 kHz, respectively. Duration and frequency of the first call in (B) is 1194 ms and 22.7 kHz, respectively. Both types of calls were emitted in short series.

Vocalization and General Behaviour Induced by Glutamate

Unilateral injection of glutamate into the anterior hypothalamic-preoptic area of freely behaving rats in a dose range of 16.9-67.6 μg (0.5-2 M solution) consistently induced behavioural agitation, exploration with characteristic short, whistle-like, high-frequency vocalizations emitted often in short series (Fig. 2A). Animals seemed to be restless, often reared, and sometimes climbed the walls, biting or pulling down the foam innerpadding of the cage. Jumping, however, was not observed in any of the animals.

Glutamate-induced ultrasonic vocalization appeared after an average latency of 34.0 ± 12.2 s (SEM, n = 16, minimum 2 s, maximum 180 s), and lasted an average of 8.1 ± 1.3 s (n = 11, minimum 3 min, maximum 17 min). Some responses started immediately and were so brief that we could not measure their latency or duration. The average emitted sound frequency for 2 M glutamate was 46.7 ± 0.46 kHz (n = 413) with minimum and maximum values of 20 and 70 kHz, respectively. Single ultrasonic calls induced by 2 M glutamate had an average duration of 47.3 ± 0.05 ms (n = 414). Some of these calls had a short rising frequency sweep (Fig. 2A).

As tested by a Mini-2 bat detector, rats did not vocalize during handling or injection procedures.

Dose-Response Study

The effects of three doses of glutamate injected unilaterally into the anterior hypothalamic-preoptic area on the magnitude of vocalization are shown in Fig. 3. The average number of calls/5 min and the number of responding rats (expressed as proportion of responders) increased with the dose of glutamate. Although only five of nine rats responded to 0.5 M glutamate, all nine rats vocalized after injection of 2 M glutamate. Friedman's ANOVA revealed significant differences among average number of calls for different doses of glutamate, $F(3, 8) = 10.2$, $p < 0.005$ (Fig. 3, filled circles). The number of calls after 0.5 and 1 M glutamate did not differ from that after control saline injection; however, 2 M glutamate caused significantly higher number of responses [Wilcoxon, $W(9) = 0$, $p < 0.007$]. There was also a significant relationship between the glutamate dosage and the proportion of responding rats. The number of responding rats was positively correlated with the dosage (Spearman's rank test, $r = 0.99$, $n = 4$, $p < 0.01$; the perfect correlation refers to ranks only).

The dose-response relationship was also studied for a number of acoustic parameters of the vocalization. The mean sound frequency after injection of glutamate was between 34.7 ± 0.12 kHz for 0.5 M glutamate and 46.75 ± 0.02 kHz (± SEM) for 2 M glutamate. The sound frequency showed a significant, positive correlation with the glutamate dosage (Fig. 4, open squares, Spearman's $r = 0.61$, $n = 240$, $p < 0.0001$ for the first 80 calls for each dose). Thus, the higher the dose of glutamate, the higher the sound frequency.

The mean duration of a single call after injection of three doses of glutamate was between 47.3 ± 0.05 ms and 59.1 ± 0.53 ms. The duration of single calls did not seem to be dependent on the glutamate dosage, and there was no significant relationship between these parameters.

Relative sound intensity (expressed in negative dB, i.e., the smaller the negative value the louder the sound) was, on average, between −12.43 ± 0.036 dB and −29.73 ± 0.029 dB and decreased with the glutamate dosage. There was a significant correlation between relative sound intensity and glutamate dosage (Fig. 5, filled triangles, Spearman's $r = -0.68$, $n = 150$, $p < 0.0001$, for the first 50 calls for each dose). The negative sign of the correlation coefficient indicates that the higher the dose, the more negative the dB value (i.e., lower intensity).

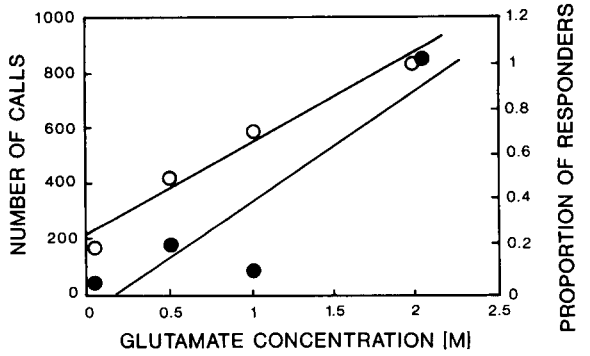

FIG. 3. Dose-response relationship between the number of calls/5 min (filled circles) as well as the proportion of responders (number of responders to total number of stimulated rats, open circles) and the molar concentration of glutamate ($n = 9$ rats). Doses of glutamate injected in 0.2 μl were: 16.9 μg for 0.5 M, 33.8 μg for 1 M, and 67.6 μg for 2 M solution of glutamate.

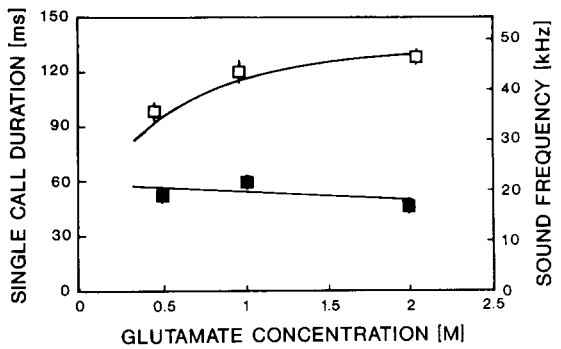

FIG. 4. Relationship between the average sound frequency/dose (in kHz, ± SEM, open squares) and the molar concentration of glutamate, and between the average single-call duration/dose (in ms, ± SEM, filled squares) and the molar concentration of glutamate. See legend to Fig. 3 for glutamate doses. Hyperbolic regression equation for sound frequency is $y = 1/(6.325 - 0.125x)$ and linear regression equation for call duration is $y = 58.955 - 4.961x$. See text for statistical results.

The average bandwidth of calls induced by three doses of glutamate was between 4.8 ± 0.04 kHz and 5.9 ± 0.026 kHz. There was no significant relationship between these parameters and the dosage.

Antagonism of the Glutamate Response by MK-801

Injection of isotonic saline caused only sporadic short calls in 2 out of 10 rats. Injection of 33.8 μg of glutamate (1 M solution) in a volume of 0.2 μl into the anterior hypothalamic-preoptic area caused a threefold increase in the number of calls and a fourfold increase in the proportion of responding rats, compared to saline control (Fig. 6). Pretreatment with

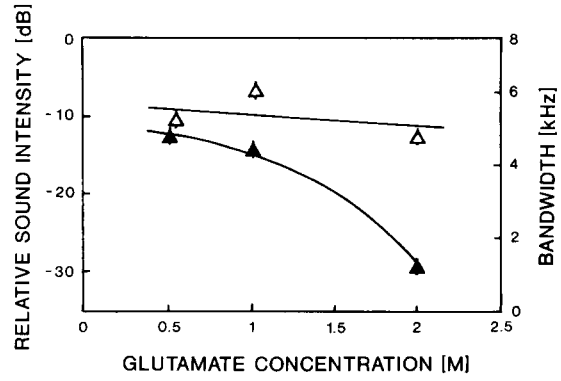

FIG. 5. Relationship between the average bandwidth/dose (in kHz, ± SEM, open triangles) and the molar concentration of glutamate, and between the average relative sound intensity/dose (in negative dB, ± SEM, filled triangles) and the molar concentration of glutamate. See legend to Fig. 3 for glutamate doses. Linear regression equation for call bandwidth is $y = 5.73 - 0.361x$ and hyperbolic regression for sound intensity is $y = 1/(-0.098 + 0.032x)$. See text for statistical results.

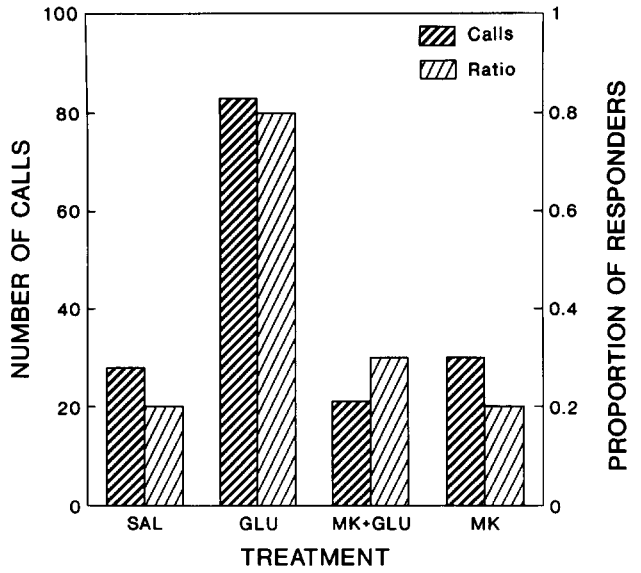

FIG. 6. Effects of pretreatment with MK-801 on glutamate-induced ultrasonic vocalization. Injection of glutamate (GLU) induced an almost threefold increase in number of short calls (heavy hatched bars) and in proportion of responders (number of responders to total number of stimulated rats, light hatched bars) in comparison to saline effects (SAL). Local pretreatment with MK-801 reversed the response (MK + GLU). Effects of injection with MK-801 alone (MK) were comparable with those after saline. See text for statistical results.

3.37 μg of MK-801 (0.05 M solution), injected in a volume of 0.2 μl into the same brain site 5 min before glutamate, reversed the effect of glutamate. Despite the clearcut response to glutamate, there were no significant differences in number of calls among all groups [Friedman's ANOVA, $F(3, 9) = 0.6$, NS) due to high number of nonresponding rats. On the other hand, number of calls induced by glutamate alone was significantly different than zero, $t(9) = 2.45$, $p < 0.04$, whereas number of calls induced by saline, glutamate pretreated with MK-801, or MK-801 alone did not differ significantly from zero, $t(8\text{-}9) = 1.4$, NS (Fig. 6).

Comparison of Glutamate- and Carbachol-Induced Ultrasonic Vocalizations

Injection of 1 μg of carbachol in a volume of 0.2 μl into the same brain sites that were injected with 1 M glutamate induced a different type of ultrasonic vocalization and behaviour from all of the brain sites studied (see Fig. 1, stars within circles).

After injection of carbachol, motor and locomotor activity of the rats were decreased. Although, the rats' motor activity was not measured, their behaviour after carbachol clearly contrasted with that after glutamate. Most of the rats after injection of carbachol resumed crouched position, were motionless, and spent most of the time vocalizing. None of the animals climbed walls or pulled down the cage innerpadding. The average sound frequency induced by carbachol was 25.47 ± 0.54 kHz (n = 165 calls, range 20.8–57.6 kHz) and average call duration was 373.78 ± 19.22 ms (n = 165, range 43–1500 ms) (Fig. 7). For comparison, 1 M glutamate, given into the same brain sites, induced calls with average sound frequency of 49.2 ± 0.67 kHz (n = 165 calls, range 19.52–72.96 kHz) and 42.47 ± 1.4 ms (n = 165, range 18–175 ms) of av-

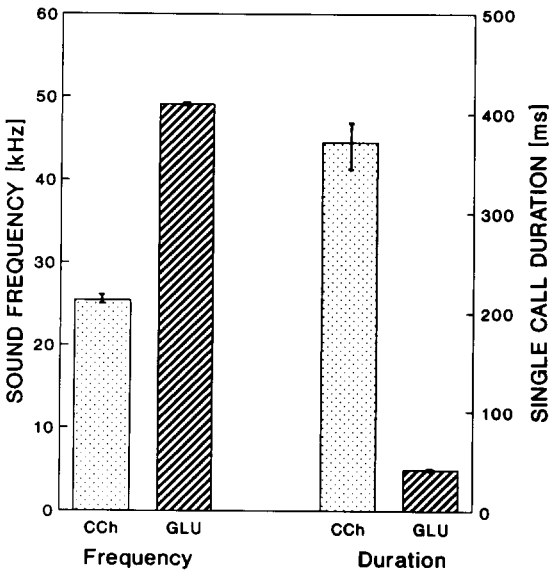

FIG. 7. Comparison of sound frequency (in kHz) and duration of calls (in ms) induced by intracerebral injection of 1 μg of carbachol (CCh, stippled columns, n = 165 calls) and 33.8 μg of glutamate (GLU, hatched columns, n = 165) given into the same brain sites. The vertical lines represent SEMs. See text for statistical results.

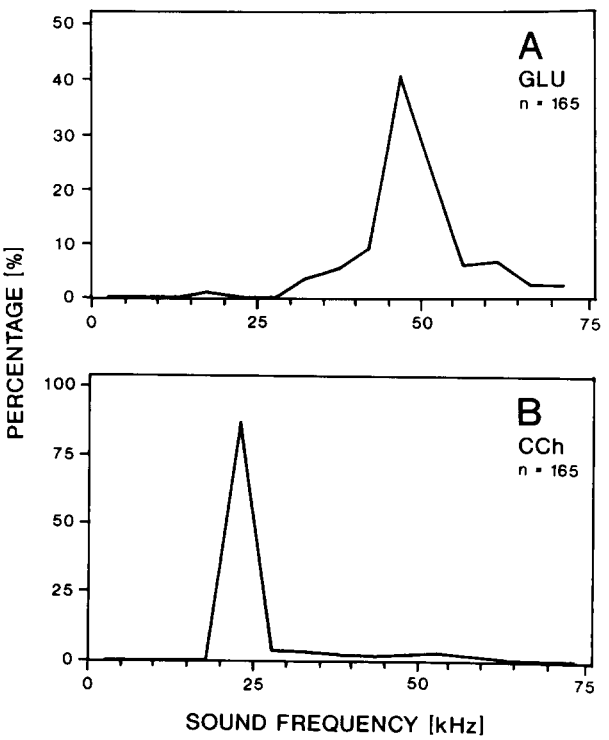

FIG. 8. Percent distributions of sound frequency (in kHz) for calls induced by intracerebral injection of 33.8 μg of glutamate (GLU, n = 165 calls in A) and 1 μg of carbachol (CCh, n = 165 in B) given into the same brain site. Width of class interval = 5 kHz. Lines connect midpoints of class intervals.

erage single-call duration. The average sound frequency and average call duration after carbachol and those after glutamate induced from the same brain sites were significantly different [t-test for correlated comparison, $t(164) = 31.3$, $p < 0.0001$ for sound frequency and $t(164) = 17.3$, $p < 0.0001$ for call duration) (Fig. 7). The distributions of sound frequencies induced by glutamate and carbachol are compared in Fig. 8A and 8B in 5-kHz bouts. The distributions are dramatically different and there is no overlap between the main peaks for these two kinds of vocalization. The distributions of single-call durations of calls induced by glutamate and carbachol are compared in Fig. 9. As shown on the combined graph in Fig. 9B, the peak of glutamate-induced calls is also distinct from that for carbachol-induced call durations. The most striking difference is that the distribution for carbachol calls is more than 10 times broader than that for glutamate calls.

DISCUSSION

Direct injection of glutamate into the anterior hypothalamic-preoptic area of the rat brain consistently induced ultrasonic vocalization from a limited forebrain region. The vocalization was characterized by high sound frequency (mostly above 40 kHz) and very short duration of single calls (below 60 ms). The glutamate-induced vocalization was dose dependent, and the increasing dosage of glutamate induced increasing number of calls and had a significant influence on the sound frequency and intensity of emitted ultrasound. The higher the dose of glutamate, the higher the sound frequency and the lower its intensity. The response was antagonized by local pretreatment with MK-801, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, and could not be induced by saline or MK-801 alone. Injection of carbachol, a muscarinic cholinomimetic agent, into the same brain sites as glutamate, induced qualitatively and quantitatively different types of calls, known as the 22-kHz vocalization.

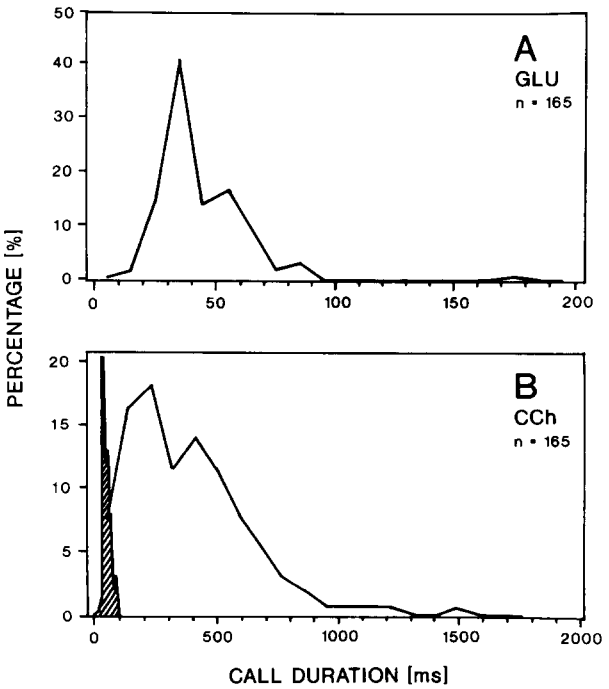

FIG. 9. Percent distributions of single-call duration (in ms) for calls induced by intracerebral injection of 33.8 μg glutamate (GLU, n = 165 calls in A) and 1 μg of carbachol (CCh, n = 165, in B) given into the same brain site. The distribution shown in (A) is redrawn in (B) as a hatched area. Width of class interval in (A) = 10 ms and in (B) = 90 ms. Lines connect midpoints of class intervals.

The glutamate-induced vocalization described in this study had similar characteristics to high-frequency (40–70 kHz) short calls emitted by rats in natural situations (2,4,20,14,18). High-frequency 40–70-kHz vocalization has been detected during overt aggressive encounters with other rats, in response to an anesthetized conspecific, after reuniting separated siblings, and during precopulatory activities (3,4,20). The ultrasounds were emitted as short pulses, 3–65 ms, frequently with marked frequency fluctuations observed as rapid sweeps. These calls were reported to correlate with aggressive behaviour and often occurred in synchrony with chasing and locomotor activity (20). The glutamate-induced calls had similar duration and frequency range, and often showed a frequency sweep. Although locomotor activity was not measured in the present study, a marked behavioural activity has also been observed after injection of glutamate.

The behaviour of rats and acoustic characteristics of their vocalization clearly contrasted with that after intracerebral injection of carbachol. Rats were inactive and remained mostly motionless, vocalizing with long 20–32-kHz calls after carbachol. Although duration of glutamate calls did not exceed 100 ms, more than 92% of carbachol-induced calls were longer than 100 ms. Similarly, the main peak of sound frequency for glutamate-induced vocalization was located at twice as high a frequency (45–50 kHz) than that for carbachol-induced calls (20–25 kHz). The carbachol-induced vocalization did not differ from that described in previous papers (5,7,11) and was similar to naturally occurring 20–32-kHz calls (6,8,11).

It is remarkable that two different types of ultrasonic vocalization, known from behavioural observations in rats and often termed 22-kHz and 50-kHz calls, were induced pharmacologically by two different agents from the same brain sites. Carbachol-induced calls could be antagonized by a specific muscarinic antagonist, atropine (5), and glutamate-induced calls by a specific NMDA receptor antagonist, MK-801, as shown in the present study. It has also been demonstrated that iontophoretic application of carbachol into the anterior hypothalamic-preoptic area had a predominantly inhibitory influence on the mean firing rate of neurons whereas glutamate, applied in the same way, exerted opposite, excitatory influence on the mean firing rate of neurons (10). These observations suggest that short-duration, high-frequency calls (50-kHz calls) and long-duration, low-frequency calls (22-kHz calls) have different neurophysiological and neurochemical mechanisms, at least at the level of the anterior hypothalamic-preoptic area. It is likely that these different mechanisms underlie different behaviours characterized by 50-or 22-kHz calls. It is suggested, therefore, that 50- and 22-kHz vocalizations may represent two different physiological states with different neural mechanisms.

ACKNOWLEDGEMENTS

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