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Pharmacology, Biochemistry and Behavior 70 (2001) 317–323

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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The related roles of dopamine and glutamate in the initiation of 50-kHz ultrasonic calls in adult rats

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Received 16 January 2001; received in revised form 25 May 2001; accepted 29 June 2001

Abstract

Effects of amphetamine on the production of 50-kHz ultrasonic calls were studied. Calls were emitted spontaneously or were induced by an intrahypothalamic–preoptic injection of glutamate. Sonographic analysis of recorded calls revealed that they were within the 35–70-kHz sound frequency range reported for the 50-kHz call type. Systemic amphetamine (AMPH, 2 mg/kg) significantly increased the number of spontaneously emitted 50-kHz calls and the effect of AMPH was dose-dependent. Low dose of intracerebral glutamate (17 μg) had no additive effect on the number of AMPH-induced calls. Higher dose of intracerebral glutamate alone (34 μg) significantly increased the number of 50-kHz calls, which was completely reversed by systemic application of haloperidol (2 mg/kg), a dopamine antagonist. The results suggest that glutamate-induced or spontaneously occurring 50-kHz calls in adult rats are dependent upon dopaminergic transmission. It is postulated that this type of calls may be indicative of dopamine mediated affective state in adult rats.

Keywords: Animal communication; Ultrasonic vocalization; 50-kHz calls; Amphetamine; Dopamine; Glutamate; Haloperidol; Anterior hypothalamic–preoptic area; Rat

1. Introduction

Ultrasonic vocalizations are emitted by rats in a number of behavioral situations and are thought to reflect the emotional and/or motivational state of the rats (Bialy et al., 2000; Blanchard et al., 1991; Knutson et al., 1998; Panksepp and Burgdorf, 2000; Thomas and Barfield, 1985). The ultrasonic vocalizations typically involve two distinct call patterns in adult animals: (1) calls of an approximate sound frequency of 19–32 kHz, known as "22-kHz calls," which are emitted in aversive situations (Blanchard et al., 1991; Brudzynski and Chiu, 1995; Brudzynski and Ociepa, 1992; Brudzynski et al., 1991; Corrigan and Flannelly, 1979) and (2) calls of an approximate sound frequency of 35–70 kHz, known as "50-kHz calls," which are emitted in nonaversive, appetitive or potentially rewarding situations (Bialy et al., 2000; Blanchard et al., 1993; Burgdorf and Panksepp, 1999; Knutson et al., 1997, 1998).

The 22-kHz calls have been reported to occur in situations when the rat is confronted by a predator (Blanchard et al., 1991), in the presence of an aggressor (Corrigan and Flannelly, 1979), in the response to the initial handling of naive rats (Brudzynski and Ociepa, 1992) or in other dangerous and stress-evoking situations (Sales and Pye, 1974). The calls can also be induced by chemical stimulation with carbachol (Brudzynski, 1994), a predominantly muscarinic agonist, and are acoustically indistinguishable from naturally occurring calls (Brudzynski et al., 1991). This pharmacological response could be prevented by pretreatment with cholinergic antagonists such as scopolamine (Brudzynski and Barnabi, 1996) or atropine (Brudzynski, 1994).

Calls of the 50-kHz type are emitted during different behavioural situations, for example, when the rat is in the presence of another anaesthetized rat (Blanchard et al., 1993), in environments that have previously been associated with reward (Knutson et al., 1999), and in anticipation of various rewards such as play in young rats (Knutson et al., 1998), and food reward (Burgdorf et al., 2000). These calls

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0091-3057/01/$ – see front matter
PII: S0091-3057(01)00615-3

could also be promoted by electrical stimulation of the ventral tegmental area (Burgdorf et al., 2000) or triggered by chemical stimulation of the mediobasal anterior hypothalamic–preoptic area (HPOA) with glutamate (Fu and Brudzynski, 1994), which has excitatory effects on hypothalamic–preoptic neurons (Brudzynski et al., 1998). The glutamate-induced response could be reversed by pretreatment with glutamate antagonist, MK-801 (Fu and Brudzynski, 1994). The 50-kHz calls were also reported to be induced by direct administration of D-amphetamine (AMPH) into the nucleus accumbens (Burgdorf and Panksepp, 1999), the drug which increases synaptic dopamine and potentiates dopamine effects.

The association between dopamine and 50-kHz calls was suggested by experiments showing that rats vocalized more in an experimental chamber that was associated with the rewarding effects of previously administered AMPH, than in the control area (Knutson et al., 1999). Also, it has been briefly reported that AMPH increased the number of 50-kHz calls when administered systemically (Knutson et al., 1997) and when injected directly into the nucleus accumbens (Burgdorf and Panksepp, 1999), however, these results have not yet been fully described. It is important to notice that recording of calls falling within the 50-kHz category in the studies associating dopamine function and vocalization, were done by a general sound frequency filtering and not by the sonographic analysis of acoustic features. It would be important to know at least an average sound frequency of these calls.

Because 50-kHz calls are emitted during potentially rewarding and appetitive situations, the mesolimbic dopamine system, which is a predominant factor in the reward hypothesis (Fibiger and Phillips, 1987; Ikemoto and Panksepp, 1999), may be a major system involved in the production of these calls. Thus, research has been focused on providing evidence to support the role of the mesolimbic dopamine in the production of 50-kHz calls.

The present study was an attempt to provide documentation of the relationship between glutamate and dopamine in producing 50-kHz calls by examining the influence of brain dopamine manipulations on the number of spontaneously or glutamate-induced calls from the AHPOA. Glutamate has been previously shown to elicit 50-kHz calls when injected directly into the AHPOA in a dose-dependent fashion (Fu and Brudzynski, 1994). It was hypothesized that administration of AMPH prior to glutamate would augment the number of 50-kHz calls produced by rats, and that haloperidol would antagonize the effects of glutamate. Pretreatment with AMPH is expected to increase dopaminergic transmission and this increase is anticipated to potentiate glutamate effects, while systemic haloperidol would antagonize dopamine effects. Since AMPH might have a powerful effect on vocalization (Knutson et al., 1999), a low, near-threshold dose of glutamate (17 μg) had been chosen for the combined experiment. Furthermore, samples of ultrasonic calls from all vocalizing animals were analyzed sonographically to provide the acoustic characteristics of their calls.

2. Method

2.1. Animals and surgeries

Fifty-one adult male Wistar rats were used in this study (Charles River, Montreal, QC). Fourty-one rats underwent stereotaxic surgery at which time they weighed between 190 and 300 g. Ten nonoperated rats were used for systemic application of amphetamine alone. The animals were housed in clear plastic cages (24 × 45.4 × 20 cm high) with a 12:12 h light/dark cycle with food and water available ad libitum. They were kept in pairs before surgery and placed in single cages afterward.

The animals received 0.01 mg/kg sc of buprenorphine (Rickett and Colman Pharmaceuticals, Hull, England) as an analgesic and were anaesthetized with a mixture injection of ketamine hydrochloride (Ayerst Veterinarian Laboratories, Guelf, ON, 40–60 mg/kg ip) and xylazine hydrochloride (Bayer, Etobicoke, ON, 4–6 mg/kg ip). Surgeries were performed in a Kopf stereotaxic apparatus with the incisor bar positioned 3.3 mm below the ear bars. Stainless steel guide cannulae were bilaterally implanted 1 mm above the intended injection site in AHPOA using the following coordinates: A: 8.1–8.2 mm from the interaural plane, L: 0.7–0.9 mm from midline, V: 8.2–8.4 mm below the surface of the cortex according to the stereotaxic atlas by Paxinos and Watson (Paxinos and Watson, 1986). Cannulae were secured to the skull with jeweller screws and methyl methacrylate resin (Perm, Hygenic Corporation of Canada, St. Catharines, ON). Sterile stainless steel plug-pins were used to close the cannulae openings. Animals received 3 ml of warm saline intraperitoneally as fluid replacement immediately following the surgery and were given at least 7 days for recovery.

2.2. Pharmacological agents and injection procedures

AMPH (Sigma, St. Louis, MO) and monosodium L-glutamate (Sigma) were dissolved in 0.9% isotonic saline. Saline was used as a control vehicle for both AMPH and glutamate. AMPH was administered systemically at low (1.5 mg/kg ip), medium (2.0 mg/kg ip) and high (2.5 mg/kg ip) doses. Glutamate was administered intracerebrally at a lower dose of 17 μg in 0.2 μl for experiments with AMPH, and at a higher dose (34 μg in 0.3 μl) for the experiment with haloperidol. The lower dose was used (Fu and Brudzynski, 1994) to avoid a potential ceiling effect after AMPH and glutamate together. Haloperidol (Precision Biochemicals, Vancouver, BC) was dissolved in a 1% lactic acid solution and administered at a dose of 2.0 mg/kg ip. Lactic acid was used as a control vehicle for haloperidol.

All intraperitoneal injections were done in a volume of 0.15– 0.25 ml. The intracerebral injections were performed with a CR-700 Hamilton microinjector in a volume of 0.2–0.3 μl unilaterally at a rate of 10–20 nl/s. After injection, the injecting cannula was left in place for 10 s before with-

drawal. Intracerebral injections were performed once per week and not more than eight injections were done into one brain site.

2.3. Experimental design

In the first experiment, effects of systemic AMPH were studied alone. Ten nonoperated rats received a medium dose of AMPH (2 mg/kg ip) alone or vehicle injection of an equivalent volume of saline (intraperitoneal) in a counterbalanced order. The injected animal was immediately placed in the recording chamber for two successive 10-min observation bouts in order to allow AMPH to reach the target sites.

In the second experiment, effects of systemic AMPH on glutamate-induced calls were studied. Twenty-one rats surgically implanted with cannulae into the brain began the testing. AMPH was given at the three doses mentioned above 10 min prior to an intracerebral injection of glutamate into the AHPOA. Vehicle injections of equivalent volume of saline were used as controls for both AMPH (intraperitoneal) and glutamate (intracerebral) in a counterbalanced order. Immediately following the pretreatment injection, each rat was placed in the recording chamber and observed for 10 min and the number of vocalizations was recorded. After 10 min, the animal was removed from the chamber and administered the intracerebral injection, placed back in the chamber for an additional 10-min period of observation, and vocalizations were recorded.

In the third experiment, effects of systemic haloperidol on the glutamate-induced calls were studied. Twenty rats surgically implanted with cannulae completed the testing phase. Rats were administered a higher dose of glutamate (34 μg in 0.3 μl ic) pretreated with intraperitoneal haloperidol or vehicle in a counterbalanced order. A higher dose of glutamate was used because it was hypothesized that haloperidol would decrease the number of glutamate-induced calls and a ceiling effect was not expected to occur.

2.4. Recording and analysis of vocalization

The recording chamber consisted of a padded, echo-free cage (25 cm wide × 18 cm deep × 18 cm high) housed in a larger sound-resistant, ventilated and temperature-controlled cubicle (BRS/LVE Tech Serv, Beltsville, MD). Vocalizations were recorded through an ultrasonic microphone model SM-1 (Ultra Sound Advice, London, England, working range 10–180 kHz) mounted in the centre of the sidewall and connected to the S200 bat detector (QMC Instruments, London, England). Signals from the broadband output of the bat detector, with the frequency division ratio 1/16, were stored on an audiotape for later sonographic analysis. Stored vocalizations were subsequently analyzed by a sonograph (DSP Sona-Graph, Kay Elemetrics, Pine Brook, NJ) to obtain sonograms and power spectra from single calls. Further analysis included measurement of single call duration (in ms), peak sound frequency (in kHz) and bandwidth (in kHz). For other details of acoustic analyses, see Brudzynski et al. (1993). Vocalization time was determined from the recorded signals, i.e., the length of time in minutes from the onset of first vocalization to the last vocalization after which the rat did not vocalize for 2 min, or when the 10-min default time was reached for each observation period.

2.5. Histological verification

After completion of experiments, animals were sacrificed by an overdose of sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Hamilton, ON), injected with 0.1 μl of 2:1 diluted suspension of India ink intracerebrally to localize injection sites, and immediately transcardially perfused with a 10% solution of formaline. The brains were fixed in 10% formaline for at least 48 h and were sectioned on a freezing microtome equipped with a cryo-stage (Cryo-Histomat, Hacker Instruments and Industries, Fairfield, NJ) for 60–70 μm preparations, air-dried, delipidized and stained with an aqueous solution of thionine stain (Gurr, 1953). Histological preparations were analyzed under microscope and maps of injection sites were composed according to the stereotaxic atlas by (Paxinos and Watson, 1986).

2.6. Statistical analysis

Repeated measures ANOVA or dependent *t* tests were used where appropriate to test the hypothesis that AMPH increases the effect of glutamate-induced 50-kHz vocalizations by increasing the number of 50-kHz calls emitted by rats. Greenhouse–Geisser correction for nonsphericity was used when necessary. The analyses were done using SPSS computer software (SPSS, Chicago, IL).

Completely randomized ANOVA was used to test for differences in acoustic parameters among different animal groups and conditions.

Initial analyses of the raw data revealed that the variance differed by more than four times across some conditions. For the data in a form of counts (number of calls), the mean is often proportional to the variance and therefore square-root transformations of the data are recommended (Howell, 1992). The raw values were transformed for all three experiments to reduce the difference in variance. A constant value of 1 was added to all raw data prior to transforming the scores because of several cases in which there were no calls (i.e., a value of 0).

3. Results

3.1. Histological verification of intracerebral injection sites

Localization of injection sites in the AHPOA is illustrated in Fig. 1. The region activated by glutamate con-

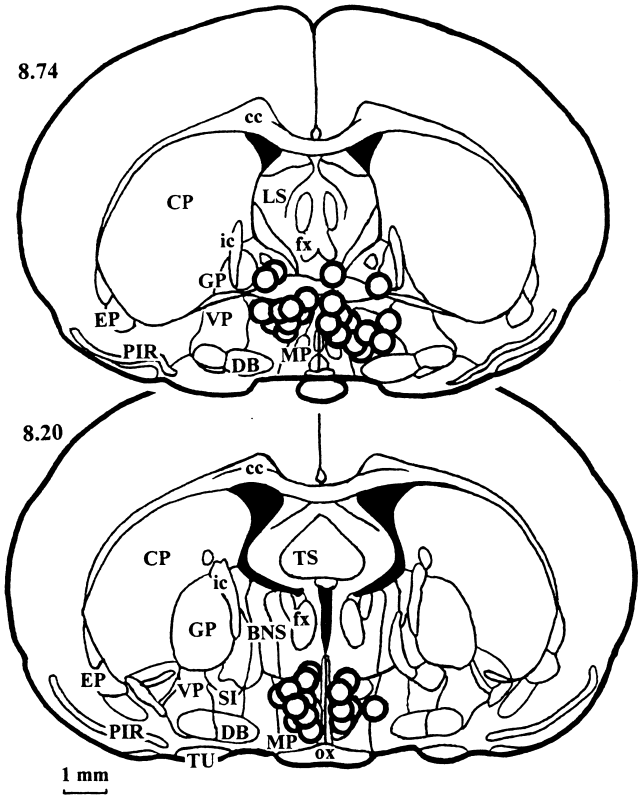

Fig. 1. Localization of intracerebral injection sites for L-glutamate (open circles). For clarity, injection sites for the lower dose of glutamate (17 μg) are marked on the left-hand side of both diagrams ($n=20$) and injection sites for 34 μg of glutamate are marked on the right-hand side ($n=18$). Numbers above the diagrams represent distance in millimeters from the interaural stereotaxic zero plane. Abbreviations: BNS—bed nucleus of stria terminalis, cc—corpus callosum, CP—caudate–putamen, DB—diagonal band (vertical limb), EP—endopiriform nucleus, fx—fornix, GP—globus pallidus, ic—capsula interna, LS—lateral septal nucleus, MP—medial preoptic area, ox—optic chiasm, PIR—piriform cortex, SI—substantia innominata, TS—triangular septal nuclues, TU—olfactory tubercle, VP—ventral pallidum.

sisted of the entire medial preoptic area, including the medial and median preoptic nuclei, anterodorsal preoptic nucleus, septohypothalamic nucleus, ventral aspect of the bed nucleus of stria terminalis, rostral aspects of the anterior hypothalamic area and periventricular nucleus and the border of the lateral preoptic area. This general region is consistent with that described previously (Fu and Brudzynski, 1994) to be sensitive to glutamate and to induce 50-kHz calls upon stimulation.

3.2. Systemic AMPH alone

Systemic injections of AMPH significantly increased the number of 50-kHz calls emitted by rats as compared to the saline control (see Fig. 2, dark bars). The effect of AMPH on the number of 50-kHz calls was examined with a Time (first vs. second 10-min bout postinjection) by Treatment (saline vs. AMPH) repeated measures ANOVA. Both a significant main effect for Time [$F(1,9)=8.830$, $P=.016$] and Treatment [$F(1,9)=18.735$, $P=.002$] were present, indicating that the AMPH induced more 50-kHz calls than saline and that there were more calls emitted during the second 10-min postinjection period than immediately following the injection. Also, a significant Treatment × Time interaction emerged [$F(1,9)=19.248$, $P=.002$], which showed that the difference between the number of calls after saline and after AMPH was greater 10-min postinjection (second 10-min bout) than immediately following the injection (Fig. 2).

Vocalization time was also examined for an effect of AMPH and the results were similar to those with the number of calls. A Treatment × Time repeated measures ANOVA revealed a significant main effect of Treatment [$F(1,9)=28.518$, $P<.001$] indicating that time spent vocalizing was longer after AMPH compared to saline. Time factor appeared not to have meaningful effect on the number of vocalization minutes. A marginally significant main effect of Time [$F(1,9)=5.00$, $P=.052$] emerged in the opposite direction, such that time spent vocalizing was slightly longer immediately following the injection, than during the second 10-min bout (Fig. 2, light bars). There was no significant interaction between Time and Treatment [$F(1,9)=0.310$, n.s.] for this parameter.

3.3. Effects of systemic AMPH on glutamate-induced response

Whereas i.p. AMPH alone had a highly significant effect on the number of calls and the number of minutes spent vocalizing (Figs. 2 and 3, AMPH-2 + SAL vs. SAL + SAL), low dose of intracerebral glutamate alone did not show any

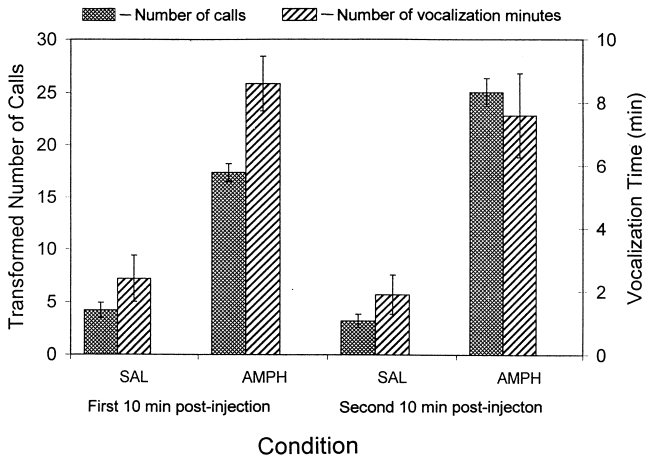

Fig. 2. The number of 50-kHz calls (transformed values) and vocalization time (i.e., the number of minutes the rat spent vocalizing in the 10-min observation time) after injection of 2 mg/kg ip of AMPH. The data are shown during the first 10 min following the injection of AMPH (first 10-min postinjection) and during the second 10-min postinjection (i.e., from 11 to 20 min after injection). Saline intraperitoneal injection (SAL) served as a control. Rats emitted more calls after AMPH than after SAL and the effect was more pronounced during the second 10-min postinjection than the first one. Vertical lines represent S.E.M. $N=10$. See text for statistical results.

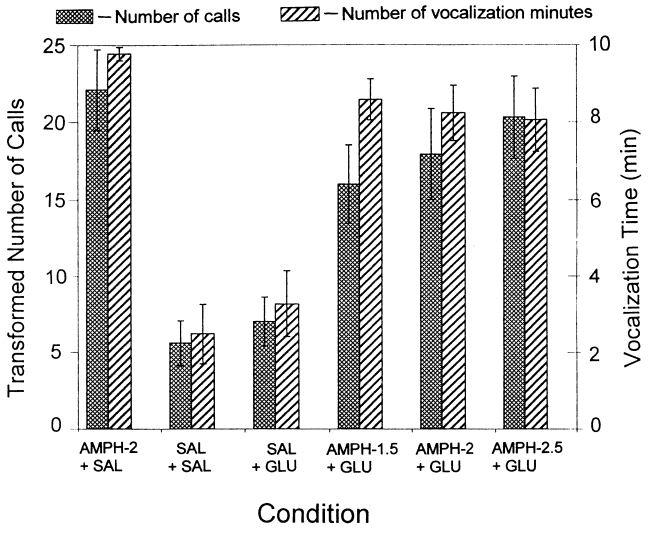

Fig. 3. The number of calls (transformed values) and number of minutes spent vocalizing (vocalization time) after intra-AHPOA (intracerebral) glutamate (GLU) pretreated with isotonic saline (SAL) or pretreated with increasing doses of amphetamine (AMPH: AMPH-1.5—1.5 mg/kg, AMPH-2—2.0 mg/kg, AMPH-2.5—2.5 mg/kg). AMPH-2+SAL represents intraperitoneal injections of 2 mg/kg of AMPH followed by intracerebral saline; SAL+SAL—intraperitoneal saline followed by intracerebral saline; SAL+GLU—intraperitoneal saline followed by 17 μg of intracerebral glutamate; AMPH+GLU—increasing doses of intraperitoneal AMPH followed by the same, 17 μg dose of intracerebral glutamate. The number of calls and vocalization minutes increased across conditions beginning from SAL+SAL. Vertical lines represent S.E.M. $N=18$. See text for statistical results.

significant effect on vocalization as compared to the effects after saline injection (Fig. 3, SAL+SAL vs. SAL+GLU).

Effects of AMPH on vocalization appeared to be dose dependent. Repeated measures ANOVA was performed on the number of calls at five levels of Treatment (Fig. 3, all bars except the first pair). A significant linear relationship emerged [$F(1,17)=30.711$, $P<.001$, $\eta^2=.644$) such that, beginning with SAL+SAL control, the number of calls increased across conditions with increasing doses of AMPH (0–2.5 mg/kg) followed by an intracerebral injection of a low dose of glutamate (AMPH+GLU). The effects of AMPH+GLU on vocalization time was similar to that with the number of calls, with a significant linear relationship [$F(1,17)=39.452$, $P<.001$, Fig. 3]. A repeated measures ANOVA was subsequently performed on only the four levels of AMPH pretreatments (Fig. 3, last four pairs of bars from SAL+GLU through AMPH-2.5+GLU). Again, a significant linear relationship emerged [$F(1,18)=20.270$, $P<.001$] with a moderately large effect size, $\eta^2=.530$, which confirmed that the linear effect was due to an increase in the number of calls with increasing doses of AMPH.

On the other hand, treatment with a low dose of intracerebral glutamate after AMPH did not show an expected additive effect. There were not more calls emitted after any of the AMPH+GLU condition as compared to AMPH effects without glutamate injection (Fig. 3, bars AMPH-2+SAL vs. any AMPH+GLU). A dependent t test was performed on the number of calls induced by the medium AMPH dose (2.0 mg/kg) pretreated with either intra-AHPOA saline or glutamate (Fig. 3, AMPH-2+SAL vs. ANPH-2+GLU). The results indicated that the anticipated additive effect of intra-AHPOA glutamate and systemic AMPH did not emerge and, in fact, there was a marginally nonsignificant trend in the opposite direction [$t(17)=-2.023$, $P=.059$].

3.4. Systemic haloperidol and intra-AHPOA glutamate

Haloperidol significantly reduced the number of glutamate-induced 50-kHz calls (Fig. 4). A Treatment (haloperidol vs. control vehicle) by Time (before glutamate vs. after glutamate) mixed ANOVA showed a significant main effect for Treatment [$F(1,18)=9.024$, $P=.008$] and Time [$F(1,18)=7.362$, $P=.014$] and a significant interaction between Time and Treatment [$F(1,18)=10.783$, $P=.004$]. Thus, this dose of glutamate caused a significant increase

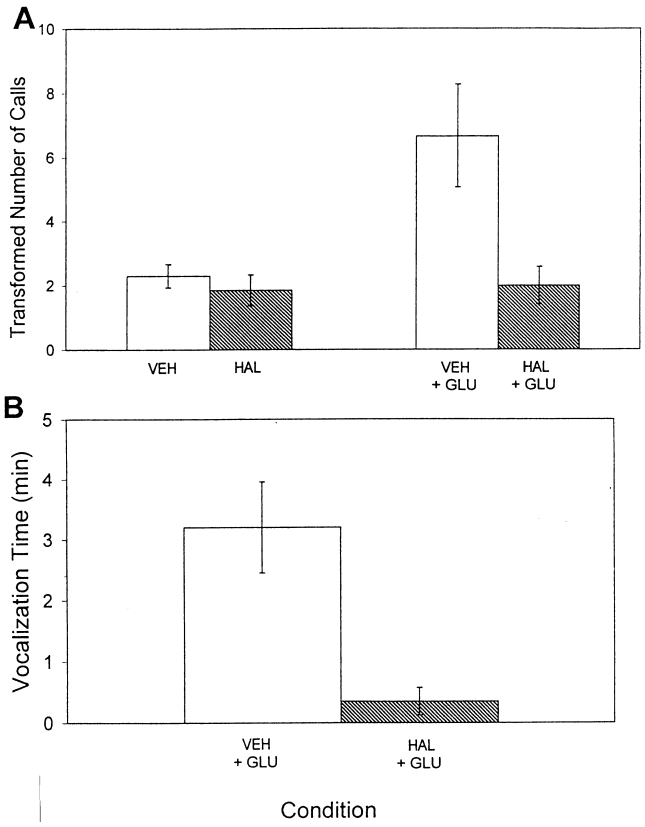

Fig. 4. (A) The number of calls (transformed values) emitted after control intraperitoneal vehicle injection (VEH), intraperitoneal haloperidol injection (HAL) alone or as a pretreatment before intra-AHPOA injection of 34 μg of glutamate (GLU). Haloperidol reversed the glutamate-induced increase in number of calls. (B) Changes in vocalization time (number of vocalization minutes) after intra-AHPOA injection of 34 μg of glutamate pretreated with VEH ot HAL. Haloperidol reversed the glutamate-induced increase in vocalization time. Vertical lines represent S.E.M. $N=20$. See text for statistical results.

in the number of calls compared to the control, and haloperidol caused a significant reversal of the glutamate effect (Fig. 4A). The vocalization time data were only available for conditions after the administration of glutamate (Fig. 4B). A dependent *t* test indicated that vocalization time (i.e., number of minutes spent vocalizing) was shorter when glutamate was pretreated with haloperidol compared to pretreatment with control vehicle [$t(9)=3.98$, $P=.001$].

3.5. Acoustic features of 50-kHz calls across conditions

An average peak frequency for spontaneously emitted calls was 54.6 ± 1.3 (S.E.M.) kHz and did not differ significantly neither from peak sound frequency of AMPH induced calls (55.1 ± 0.9 kHz, for 2 mg/kg) nor from that of glutamate induced calls (55.0 ± 1.5 kHz for 34 μg, see Fig. 5). Analysis of variance confirmed lack of significant differences among different animal groups and ways of vocalization induction [$F(4,80)=0.55$, n.s.]. Similarly, there was no significant difference in duration of individual 50 kHz calls emitted spontaneously, or by AMPH, or glutamate (Fig. 5). The average duration of single call was 32.4 ± 1.8 ms for spontaneous calls, 37.5 ± 1.8 ms for 2 mg/kg of AMPH, and 33.9 ± 3.2 ms for 34 μg of glutamate. ANOVA has confirmed lack of significant differences among different animal groups and ways of vocalization induction [$F(4,80)=0.91$, n.s.].

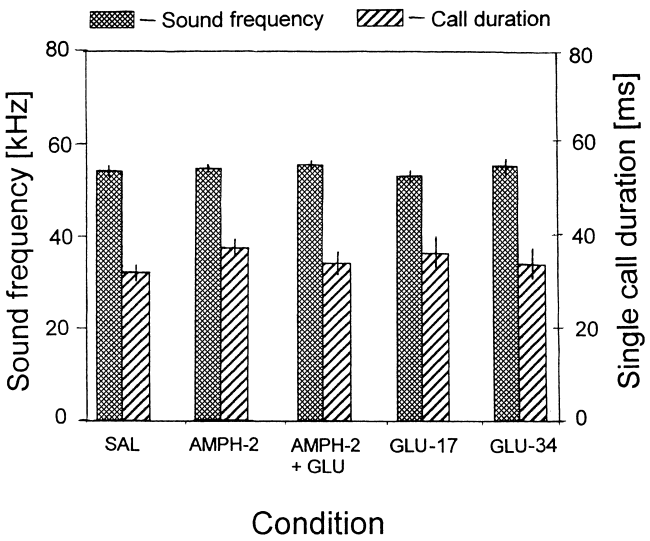

Fig. 5. Average peak sound frequency (cross-hatched bars) and average duration of a single 50-kHz call (hatched bars) in different experimental conditions: SAL—parameters of spontaneously appearing 50-kHz calls after intraperitoneal or intracerebral injection of isotonic saline ($N=18$); AMPH-2—parameters of amphetamine-induced (2 mg/kg ip) calls ($N=23$); AMPH-2+GLU—parameters of calls induced after glutamate (17 μg ic) pretreated with 2 mg/kg intraperitoneal amphetamine ($N=20$); GLU-17—parameters of 50-kHz calls emitted after intracerebral injection of 17 μg of glutamate with intraperitoneal saline pretreatment ($N=12$); GLU-34—parameters of 50-kHz calls emitted after intracerebral injection of 34 μg of glutamate with intraperitoneal saline pretreatment ($N=12$). Vertical bars represent S.E.M. See text for statistical results.

Acoustic parameters of all emitted calls remained unchanged regardless of the conditions and were within the typical ranges for sound frequency and call duration for adult rats.

4. Discussion

Our results have indicated that systemic AMPH increased the number of 50 kHz calls emitted by adult male rats. However, when the combined effects of both increasing doses of systemic AMPH and a low, constant dose of intra-AHPOA glutamate were examined, an additive effect of glutamate on vocalization was not observed. The number of calls emitted after each increase in the AMPH dose (with a constant dose of glutamate) did increase in a linear, dose-dependent fashion. On the other hand, the higher dose of glutamate alone significantly increased the number of 50-kHz calls. Moreover, the glutamate-induced calls were reduced to the control level when rats were pretreated with systemic haloperidol, a dopamine receptor antagonist. The increased amount of time rats spent vocalizing after the drug treatment was consistent with the increased number of calls per time unit.

The results presented here support the hypothesis that dopamine and glutamate are both involved in the production of 50-kHz calls of adult rats. Both the glutamate and AMPH effects are dose dependent (Fu and Brudzynski, 1994 and the present study). Calls induced by intracerebral glutamate, by combined AMPH and glutamate application, or by AMPH alone, all have acoustic features which are fully comparable with naturally occurring 50-kHz calls, i.e., 35–70 kHz sound frequency and short call duration of less than 100 ms (Blanchard et al., 1993 and present data). Our result that AMPH increased the number of 50-kHz calls (Fig. 1) and data from other reports that 50-kHz calls could be induced by AMPH injections into the nucleus accumbens (Burgdorf and Panksepp, 1999; Burgdorf et al., 2000), and that 50-kHz calls occur in anticipation of experimenter-delivered electrical stimulation to the ventral tegmental area (location of dopamine cell bodies) and lateral hypothalamus (Burgdorf et al., 2000), provide a strong argument for the role of mesolimbic dopaminergic system in the initiation of these calls. This conclusion is also consistent with other data indicating that 50-kHz calls are associated with a conditioned reward (Knutson et al., 1999; Panksepp and Burgdorf, 2000).

The glutamate-induced increase in the number of 50-kHz calls from AHPOA was reversed by systemic pretreatment with dopamine receptor antagonist, haloperidol. This result indicates not only that dopamine mediation is a necessary condition for an increased production of these calls by glutamate, but also that the AHPOA may be one of the brain sites where a direct interaction between dopamine and glutamate may occur. Such a direct interaction between dopamine and glutamate terminals have been postulated in the nucleus accumbens as a mechanism regulating motiva-

tionally initiated locomotor activity in rats (Wu and Brudzynski, 1995; Wu et al., 1993). It appears from our result that haloperidol did not block the animal's ability to produce 50-kHz calls but rather reversed the glutamate effect to the level of the spontaneously emitted calls.

Local, intra-AHPOA pretreatment with a low dose of glutamate did not show an anticipated additive effect on the number of calls produced by systemic AMPH. If glutamate acts through dopaminergic effect, then the lack of additive effects could be due to the action of glutamate and dopamine on a common inhibitory output neuron implicated in the production of 50-kHz calls. Once the neuron is inhibited, potentiation of dopamine action may remain without any further effect on that neuron and, as a consequence, on the behavioural outcome. On the other hand, the dose of glutamate could be too low or applied into an insufficient area of the target structure. This mechanism may be more complex and requires further systematic studies.

In conclusion, the results have shown that systemic AMPH and intra-AHPOA glutamate both can induce comparable 50-kHz calls in adult rats. The study provided evidence that glutamate-induced response is mediated by dopamine because systemic haloperidol completely reversed the glutamate response. Recent results from other laboratories indicate that 50-kHz calls "mark a state of reward anticipation" (Burgdorf et al., 2000) caused by intracranial electrical stimulation and may serve as a tool in "distinquishing different types of affective states in rats" (Burgdorf et al., 2000). Implication of dopamine in reward seeking behaviour (Ikemoto and Panksepp, 1999) and the mediating role of dopamine in production of 50-kHz calls support that notion and further suggest that this type of calls may be indicative of dopamine mediated affective state in adult rats.

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