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# Novel GABA Agonists Depress The Reward Effect of Lateral Hypothalamic Stimulation in Rats

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BACKUS, L. I., J. R. STELLAR, J. JACOB, G. W. HESSE AND V. E. SHASHOUA. *Novel GABA agonists depress the reward effect of lateral hypothalamic stimulation in rats.* PHARMACOL BIOCHEM BEHAV **30**(3) 657–663, 1988.—Rats were given systemic injections of one of a series of novel GABA compounds which can penetrate the blood-brain barrier to release GABA into the brain. They were then tested on lateral hypothalamic self-stimulation behavior using a rate-frequency paradigm to discriminate effects on reward from those on motor/performance. Both reward and, to a lesser extent, motor/performance impairments were found with all GABA compounds. In more extensive testing with one compound, $LG_2$, no differences in the effects of three salts (acetate, ascorbate, and tartarate) were found except that the tartarate salt effects decayed more rapidly.

GABA Novel agonists Blood-brain barrier Self-stimulation Reward Lateral hypothalamus

GAMMA-AMINO-BUTYRIC ACID (GABA) is a widely distributed inhibitory neurotransmitter in the brain [1, 5, 15, 21]. Systemic administration of certain GABA agonists produces decreases in behavioral activity [13,28], antagonizes seizure activity [18,26], and may be of therapeutic value in a wide range of behavioral and neurological disorders ranging from ethanol withdrawal [17] to Huntington's disease [4,24]. GABA itself cannot be used clinically because it does not penetrate the blood-brain barier and existing GABA agonists such as muscimol have strong toxic side effects [18,26]. One important alternative approach, which has had a wide range of therapeutic applications, has been to enhance GABA function through receptor modulation by benzodiazepines [35].

Recently, a novel class of GABA compounds have been synthesized which can penetrate the blood-brain barrier to deliver GABA to the brain [12, 13, 28]. These compounds are prepared by linking GABA to a carrier molecule which naturally penetrates the blood-brain barrier. Once inside the brain, enzyme action cleaves the GABA from the carrier molecule allowing the GABA to interact with its receptors. A number of these GABA compounds have already been shown to effectively deliver GABA to the brain and to depress behavioral activity in open field tests [12, 13, 28].

This paper describes additional studies of the biological activity of some of these GABA compounds in a test of limbic/affective function using lateral hypothalamic self-stimulation behavior in rats which has shown sensitivity to GABAergic drugs [32]. Self-stimulation behavior is known to be altered by GABA agonists and antagonists [8,25], and with modern methodologies such as the rate-frequency curve-shift method, self-stimulation studies can yield specific measures of drug-induced changes in reward and operant motor/performance functioning [2, 3, 33, 34].

In the rate-frequency method, the rate of lever pressing for rewarding brain stimulation is determined at a number of stimulation frequencies. The rate of pressing is plotted as a function of the log stimulation frequency to yield a sigmoidal curve that typically rises to an asymptotic behavioral level like a dose-response curve in pharmacology. Two statistics are derived from the rate-frequency curve; the stimulation frequency required to sustain half of the maximal responding which is termed the locus of rise (LOR), and the behavioral asymptotic maximum (MAX).

Extensive validation experiments with reductions in stimulation current and the introduction of physical obstacles have shown that lateral shifts of the rate-frequency curve, i.e., changes in LOR, signify changes in the reward effectiveness of the brain stimulation; while vertical shifts of the rate-frequency curve, i.e., changes in MAX, signify

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TABLE 1
GABA COMPOUNDS EMPLOYED IN THIS STUDY

| | |
|---|---|
| Base | $CH_2$-O-CO-$CH_2$-$CH_2$-$CH_2$-$NH_2$<br>\|<br>$CH_2$-O-CO-$CH_2$-$CH_2$-$CH_2$-$NH_2$<br>\|<br>$CH_2$-OCOR |
| Compounds | |
| $LG_2$ | 1-linolenoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol<br>R = $-(CH_2)_7(CH{=}CH{\cdot}CH_2)_3CH_3$ |
| $L^2G_2$ | 1-linoleoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol<br>R = $-(CH_2)_7(CH{=}CH{\cdot}CH_2)_2(CH_2)_3CH_3$ |
| $OG_2$ | 1-oleoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol<br>R = $-(CH_2)_7CH{=}CH{-}(CH_2)_7CH_3$ |

changes in the motor/performance capacity of the animal [3, 19, 34]. Importantly, these two statistics appear to be independent of each other in that physical obstacles which drastically impair MAX change LOR only slightly, if at all and low currents which drastically shift LOR do not change MAX [34]. Furthermore, the drug-induced changes in the stimulation-produced reward effect can be expressed quantitatively as the amount of change in stimulation frequency that is required to achieve the LOR under drug vs. under baseline conditions [3,34]. Pharmacologically, the rate-frequency method has been used extensively in medial forebrain bundle self-stimulation studies with dopamine antagonists which cause an increase in LOR and a depression of MAX, indicating both a reward an motor/performance impairment [7, 19, 30, 31]. These results are in agreement with the findings of other reward-specific behavioral methods [6, 8, 10, 33], providing important pharmacological cross-validation for the rate-frequency method.

In the first experiment with novel GABA compounds, the rate-frequency method was used to evaluate the behavioral effects of several compounds that are listed in Table 1. In the second experiment, the properties of $LG_2$, the most interesting of the compounds from Experiment 1 was further studied in its acetate, ascorbate, and tartarate salt forms.

## METHOD

### *Subjects and Surgery*

Twelve male Sprague-Dawley rats were obtained from the Charles River Breeding Labs (Wilmington, MA) and used in these experiments (7 rats in Experiment 1 and 5 in Experiment 2). All subjects were housed singly in plastic tubs 46 cm deep × 20 cm wide × 22 cm high, with food and water continuously available on a wire rack top. The animal colony was maintained on a 12-12 hour day-night reversed cycle with controlled temperature (22±2°C) and humidity (>50%). All rats were tested during the dark phase of their day-night cycle.

At the time of surgery, rats weighed between 287 and 555 grams. Under Nembutal anesthesia (55 mg/kg), each rat was stereotaxically implanted with a monopolar, stainless steel electrode constructed from "00" gauge insect pins and insulated with Formvar enamel to within 0.5 to 0.25 mm of the tip. The electrodes were aimed at the medial forebrain bundle at the level of the lateral hypothalamus. The level-skull bregma-based stereotaxic coordinates for all rats were: A.P. −3.0 mm, M.L. +1.7 mm, DV −7.5 mm (cortex). Electrodes were attached to Plastic Produce Co. connectors, and anchored with dental acrylic molded around four stainless steel screws attached to the skull. The electrode's ground wire wrapped around two of the skull screws served as the return path for the current passed through the electrode.

### *Self-Stimulation Apparatus*

Self-stimulation testing was conducted in a standard 23×21×21 cm Plexiglas operant chamber with a metal rung floor and a metal lever centered 4 cm above the floor on one wall. A house light was mounted in the ceiling, and a reinforcement light was mounted adjacent to the lever. The operant chamber was enclosed in a ventilated wooden box to attenuate sound from the laboratory environment. Electrical brain stimulation was delivered through a two channel commutator that entered the operant chamber through a hole in the ceiling and that mated with the connector on the rat's head, allowing freedom of movement within the operant chamber. Brain stimulation consisted of cathodal, monophasic square-wave pulses of 0.1 msec duration delivered from a constant current source that maintained a low resistance shunt during the interpulse interval. Stimulation levels were monitored on an oscilloscope. Control of the operant chamber and measurement of lever pressing rate was accomplished through the use of a Timex-Sinclair microcomputer and Byte-Back Co. interface.

### *Self-Stimulation Procedure*

Four days after surgery, rats were screened for self-stimulation on a continuous reinforcement schedule where reinforcement was a 0.5 second burst of 100 Hz pulses. Current was initially set at 100 $\mu$A and raised during screening to establish vigorous self-stimulation behavior with minimal aversive or motoric side effects. The maximum current employed in this study was 600 $\mu$A.

Following the establishment of self-stimulation behavior, rats were trained in an extinction/reacquisition procedure where reinforcement conditions alternated every 90 seconds between a stimulation pulse frequency of 1 Hz and 100 Hz. All reinforcement conditions were accompanied by house

TABLE 2

REWARD EFFECTS OF DRUGS BY TIME, DRUG, AND SUBJECT AS DERIVED FROM LOR (NATURAL Hz SCALE) DIFFERENCES FROM NO-DRUG BASELINE

| Drug and Dose (mg/kg) | Minutes Postinjection 0 | 20 | 40 | 60 | 80 | 100 | 120 | Animal |
|---|---|---|---|---|---|---|---|---|
| | % Change Reward Effectiveness | | | | | | | |
| $LG_2$ Acetate | | | | | | | | |
| 8 | — | — | +09 | −07 | +17 | +11 | +07 | KW8 |
| 8 | −00 | −02 | −05 | +02 | −05 | −09 | — | LB3 |
| 13 | −02 | −32* | +09 | +11 | −05 | +09 | +07 | KW8 |
| 13 | −54† | −37† | −33† | −32† | −22* | −07 | +02 | LB3 |
| 26 | NA | −38† | −40* | −45* | −44* | −29* | −00 | LB1 |
| 26 | NA | −02 | −17* | −15 | −15* | −00 | −15 | LB2 |
| 26 | −55* | −60* | −48* | −34† | −34† | +05 | −00 | LB3 |
| 26 | −31 | −35* | −31* | −31* | −24 | −28* | −32* | KW8 |
| 26 | −09 | −02 | −05 | −07 | −02 | −02 | −07 | LK18 |
| $L^2G_2$ | | | | | | | | |
| 25 | −32† | −00 | +07 | +07 | +09 | — | — | KW11 |
| 50 | −31 | −37 | −37 | −28 | −28 | — | — | KW12 |
| 100 | −34† | −32† | −32† | −32† | −32† | −38† | −31† | KW11 |
| 100 | +05 | −24 | +02 | −09 | +09 | −00 | −31† | KW12 |
| $OG_2$ | | | | | | | | |
| 30 | −05 | −09 | −21† | −24† | −11 | −09 | — | KW11 |
| 55 | −31† | +02 | −00 | +05 | −09 | — | — | KW11 |
| 55 | +24 | +19 | +19 | −07 | +05 | — | — | KW12 |
| 100 | −32† | −29† | −32† | −31† | −26* | −28* | −26* | KW11 |
| 100 | −17 | −22 | −22 | −21 | −34 | −31 | −24 | KW12 |

*†Indicate that the differences were 2.0–4.0 or >4.0 baseline standard deviations, respectively. NA indicates LOR could not be calculated due to lack of responding. —Indicates rate-frequency curve was not run.

light illumination and followed by a 5 second period of house light blackout to indicate the change to a new pulse frequency. During the blackout period pressing the lever did not result in stimulation and any responses were not counted. The reinforcement light was illuminated concurrently with the delivery of the burst of stimulation pulses. Rats were given up to 10 alternations of pulse frequency per day and extinction training was terminated when the rat showed rapid extinction or reacquisition within the first 30 seconds of the condition.

Rats were then placed in the rate-frequency procedure where the stimulation pulse frequency conditions varied systematically throughout a session, starting from a high value of 158 Hz (i.e., 2.2 log units) and descending in 0.2 log unit steps to a low of 25 Hz (1.4 log units). The duration of the conditions and blackouts, and the operation of the reinforcement light were as above. Schedule time was not accumulated and lever responses were not counted during the 0.5 seconds of each stimulation delivery to minimize data contamination by stimulation-elicited responses. Data from the first 30 seconds of each frequency condition were set aside to allow the rat's responding to adjust to the frequency change. Response rate during the last 60 seconds was used to generate the rate-frequency curve.

Rate-frequency procedures took about 9 minutes to run and were repeated with a periodicity of 20 minutes. One day's testing session consisted of 6 frequency sweeps, lasting about 2 hours. Several daily testing sessions were run for training and for any final adjustments in stimulating current. Daily sessions were then run until the two rate-frequency parameters, LOR and MAX (see the Introduction) stabilized. Stability was achieved when no trend was observed in these two parameters, the variation of LOR was within a range of 0.1 log units, and the variation for MAX was less than 20%. Four baseline sessions were then collected and drug trials begun. Baseline sessions were collected on the days between each drug test day to insure the animal had returned to normal. If baseline LOR and MAX stability was not preserved after a drug test, additional baseline days were run to achieve stability and the preceding baseline days were not included in the final baseline calculation. Between 8–10 baseline days were collected for each rat.

*Drug Administration*

In the first experiment, the GABA compounds (i.e., drugs) employed are listed in Table 1. Drugs and doses were administered in random order, but each animal did not receive all drugs. Drugs were delivered IP just before rate-frequency testing, and volume injected varied between 0.1 and 1.0 cc to accommodate dose and drug concentration. All drugs in the first experiment were dissolved in a vehicle of 20% polyethylene glycol in 0.9% saline, which by itself was shown to have no effect on the rate-frequency curve in pilot testing (3 subjects, each with LOR and MAX shifts of <10% after

TABLE 3

MOTOR/PERFORMANCE EFFECTS OF DRUGS BY TIME, DRUG, AND SUBJECT AS DERIVED FROM MAX DIFFERENCES FROM NO-DRUG BASELINE

| Drug and Dose (mg/kg) | Minutes Postinjection 0 | 20 | 40 | 60 | 80 | 100 | 120 | Animal |
|---|---|---|---|---|---|---|---|---|
| | % Change in Performance | | | | | | | |
| $LG_2$ Acetate | | | | | | | | |
| 8 | — | — | −35 | −19 | −12 | −26 | −6 | KW8 |
| 8 | −2 | −4 | 0 | +20 | −4 | +2 | — | LB3 |
| 13 | −8 | −45 | −38 | −36 | −36 | −27 | −2 | KW8 |
| 13 | −67* | −25 | −17 | −6 | −26 | −22 | −8 | LB3 |
| 26 | −100* | −56* | −63* | −42 | −75 | −87* | −54 | LB1 |
| 26 | −100† | −38* | −4 | −31* | −24 | −28 | −20 | LB2 |
| 26 | −52 | −34 | −28 | −21 | −59 | −25 | −17 | LB3 |
| 26 | −14 | −17 | −26 | −38 | −42 | −31 | −27 | KW8 |
| 26 | +53 | +1 | +4 | −11 | +1 | −11 | −26 | LK18 |
| $L^2G_2$ | | | | | | | | |
| 25 | 28* | −14 | −5 | −12 | −15 | — | — | KW11 |
| 50 | 0 | −7 | −22 | −20 | −15 | — | — | KW12 |
| 100 | −18 | −51† | −56† | −55† | −67† | −71† | −70† | KW11 |
| 100 | +15 | +4 | +9 | +8 | −21 | −24 | −6 | KW12 |
| $OG_2$ | | | | | | | | |
| 30 | −19 | −36† | −24* | −7 | −15 | −16 | — | KW11 |
| 55 | +11 | −17 | −17 | −11 | −10 | — | — | KW11 |
| 55 | −33 | −39 | −24 | −18 | −28 | — | — | KW12 |
| 100 | −21 | −43* | −39* | −31* | −39* | -34 | −32* | KW11 |
| 100 | −27 | −55* | −46 | −46 | −42* | −42 | −28 | KW12 |

*†Indicate that the differences were 2.0–4.0 or >4.0 baseline standard deviations, respectively. —Indicates rate-frequency curve was not run.

vehicle alone). In the second experiment, each rat was given two doses (13 and 20 mg/kg) of the 3 $LG_2$ salts (acetate, ascorbate, and tartarate), administered in a random fashion with respect to dose and salt type. In Experiment 2, all drugs were dissolved in 0.9% saline as the vehicle.

*Analysis of Rate-Frequency Data*

In the data analysis, LOR and MAX were separately averaged for each of the 6 rate-frequency sessions run every 20 minutes on each baseline day. In every subject, differences were calculated in log Hz between the mean baseline LOR at each time interval and the individual LOR of each drug trial at each time interval. LOR scores are presented as shifts in log Hz or as percent change in reward effectiveness of the stimulation where a drug-induced increase of 0.3 log Hz in LOR, which represents a doubling of required stimulation frequency, is taken as a 50% decrease in lateral hypothalamic reward pulse efficiency [31,34]. MAX scores of the rate-frequency curve were analyzed in the same fashion as described for LOR. MAX scores under drug conditions are presented as percent of baseline.

*Histology*

Following the completion of self-stimulation testing, rats were surgically anesthetized with Nembutal, perfused transcardially with isotonic saline and 10% formal saline. Brains were removed, stored for at least 1 week in formalin, for 1 day in 20% sucrose formalin, and cut into 40 micron slices on a cryostat at −10°C. Sections were mounted on slides, stained with cresyl violet, and cover-slipped. Electrode tip location was plotted on plates from the atlas of Paxinos and Watson [23].

## RESULTS

In Experiment One, all GABA compounds that significantly altered brain stimulation reward did so, in part, by depressing reward function (Table 2). The magnitude of the drug-induced decrease in reward that is displayed in Table 2 is derived directly from the increase in stimulation frequency (natural scale) required to reach LOR, as explained above. $LG_2$ had the largest effects at the lowest dose with initial reward decreases exceeding 40% in two subjects, and in one subject at two doses. No other subject or drug tested produced this level of depression in stimulation reward effectiveness. In 3 of 5 subjects receiving $LG_2$, a decrease on the order of 35–50% was seen in the efficiency of stimulating pulses in generating the self-stimulation reward effect (or about 0.2–0.3 log Hz increase in LOR). From Table 2 it can also be seen that at 26 mg/kg, $LG_2$ produced reward depressions that were greater than 2 baseline standard deviations in 4 of 5 subjects. These $LG_2$ effects lasted about 100 minutes. The other compounds: $OG_2$, $L^2G_2$, showed some indication of effectivness but at higher doses or under limited testing.

As seen in Table 3, the GABA compounds tested in Experiment One produced decreases in MAX, but in terms of

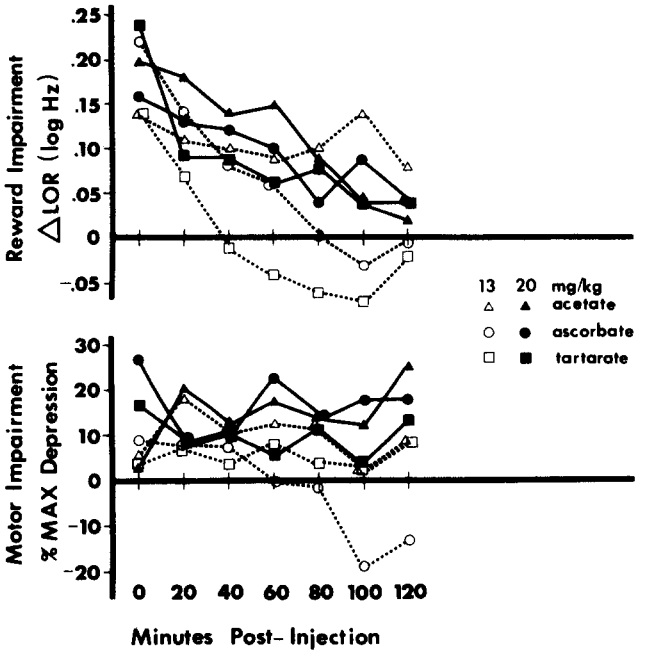


FIG. 1. Time course of reward and motor/performance impairments following $LG_2$ salts of acetate, ascorbate, or tartarate. Plotted points are means of differences from baseline performance with an average standard error term of 0.05 log units for LOR, and 7% for MAX. Note that reward impairment grows with increasing positive LOR difference and that a 0.1, 0.2 or 0.3 log unit increase in LOR represents a 21, 37%, or 50% decrease in reward, respectively. Motor/performance impairment increases with increasing percent MAX depression.

standard deviations, MAX decreases were smaller than those seen with LOR. For example, at 26 mg/kg $LG_2$ (Tables 2 and 3) reward effects (LOR) of the drug more than twice as often exceeded statistical criterion as compared to motor effects (MAX) of the drug in the same subjects. Note: each entry in Table 2 and 3 refers to an individual baseline mean and standard deviation upon which the statistical criterion were based. In Table 3, occasional instances of naturally high variability prevented some cases of large magnitude shifts from meeting the statistical criterion. Comparison of the magnitude of reward and motor shifts (Tables 2 and 3) is not meaningful because these measures represent fundamentally different behavioral processes.

For Experiment 2, which concentrated on the effects of $LG_2$ in the form of differing salts, the results for the LOR and MAX are given in Fig. 1 for the groups receiving acetate, ascorbate, and tartartate salts of $LG_2$. In Fig. 1, LOR increases indicate reward decreases. A drug-induced increase of 0.2 log units in LOR increases represents a 37% decrease in stimulation reward effectiveness. Time-dependent effects were seen with all salts in LOR scores in Fig. 1, which agrees with previous observations (Table 2). In Fig. 1, the tartarate salt had the fastest decay in effect of reward depression and even moved nonsignificantly into the area of reward increases, after 40 minutes. No evidence of rapid decay in MAX scores was seen in Fig. 1. Statistical information on the data presented in Fig. 1 is displayed in Table 4 which shows the % of subjects in each group exceeding the statistical criterion. In Table 4, MAX scores are less often significantly different from baseline than LOR scores and the incidence of exceeding statistical criteria drops with time, supporting the conclusions about time course drawn from Fig. 1. Among the $LG_2$ salts, tartarate appears to have the fastest decay of the LOR effect.

TABLE 4

PERCENT OF SUBJECTS WITH LOR OR MAX SCORES EXCEEDING STATISTICAL CRITERION* IN $LG_2$ SALT TEST (FIGURE 1)

| Salt | Dose (mg/kg) | Minutes Postinjection 0 | 20 | 40 | 60 | 80 | 100 | 120 |
|---|---|---|---|---|---|---|---|---|
| | | LOR Scores (Reward Effects) | | | | | | |
| Acetate | 13 | 40 | 20 | 40 | 20 | 20 | 20 | 20 |
| | 20 | 75 | 50 | 75 | 75 | 50 | 0 | 25 |
| Ascorbate | 13 | 80 | 60 | 40 | 20 | 0 | 0 | 0 |
| | 20 | 75 | 50 | 75 | 50 | 25 | 50 | 25 |
| Tartarate | 13 | 60 | 40 | 0 | 0 | 20 | 20 | 0 |
| | 20 | 100 | 25 | 50 | 50 | 50 | 25 | 25 |
| | | MAX Scores (Motor/Performance Effects) | | | | | | |
| Acetate | 13 | 20 | 20 | 0 | 0 | 20 | 0 | 0 |
| | 20 | 0 | 25 | 25 | 0 | 25 | 25 | 50 |
| Ascorbate | 13 | 20 | 0 | 0 | 0 | 20 | 20 | 20 |
| | 20 | 50 | 25 | 0 | 25 | 0 | 25 | 25 |
| Tartarate | 13 | 20 | 20 | 40 | 0 | 20 | 0 | 0 |
| | 20 | 50 | 25 | 0 | 0 | 25 | 0 | 25 |

*Statistical criterion employed a $t$-statistic to establish modified 95% confidence limits around the baseline means for LOR and MAX, as previously published [31].

The electrode placements for subjects in Experiment 1 appear in Fig. 2. Electrode localizations in both experiments confirmed placement for subjects within the medial forebrain bundle at the level of the lateral hypothalamus.

## DISCUSSION

Taken together, the results of these two experiments show the $LG_2$, which is known to enter the brain and releases GABA [13,28], has behavioral effects on a motivated behavior, electrical self-stimulation of the medial forebrain bundle. Second, the nature of the effects on stimulation-produced reward are very clearly to impair the stimulation-produced reward effect and to a much lesser extent, the operant motor/performance capacity.

The size and pattern of these $LG_2$ effects on the reward and motor/performance components of self-stimulation is reminiscent of our findings with midrange doses of the neuroleptic pimozide [31]. Such a comparison with the action of a neuroleptic may be possible because of the known inhibitory effect of GABA on the dopamine containing cell bodies in the ventral tegmental area and substantia nigra pars compacta. For example, it has been found that direct infusion of GABA in these areas inhibits cell firing and dopamine release [36,37] while direct infusions of GABA antagonists in-

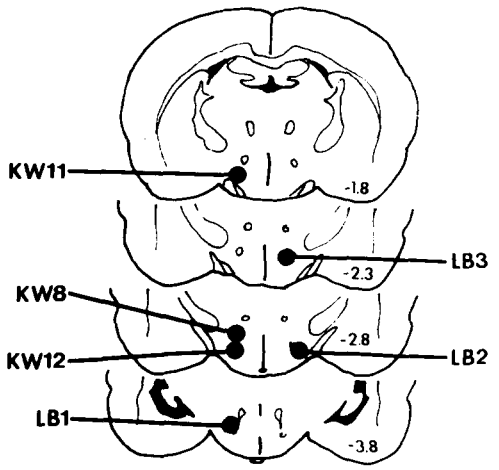


FIG. 2. Electrode tip locations are shown on plates from the atlas of Paxinos and Watson [23]. Distance in millimeters posterior to bregma is indicated on each plate. Data from rat LK18 was lost in histological processing.

creases dopaminergic release, increasing behavioral activity [21,22]. However, GABA is distributed throughout the brain [5,27], including dopamine terminal fields, such as the nucleus accumbens which is highly relevant to self-stimulation reward [30]. Also, GABA is thought to play a prominent role in some accumbens outputs [20]. However, in the accumbens or elsewhere, GABA could act on the stimulation-produced reward signal in a dopamine-independent manner.

In other self-stimulation experiments using GABA antagonists, primarily picrotoxin, self-stimulation behavior and stimulation-produced reward was attenuated [14, 25, 29, 38]. Therefore, one might wonder why we did not find the result of stimulation-produced reward enhancement with GABA angonistic coumpounds. One of these picrotoxin studies [14] is difficult to interpret because it measured self-stimulation through lever-pressing response rate at a single level of stimulation parameters. It is now recognized that this measure is nonspecific for reward due to a basic confound with motor/performance effects of drugs [19,32]. The fact that picrotoxin can produce motor/performance debilitating seizures in combination with brain stimulation, is indicated by its recent use as a motor debilitating agent in an experiment designed to validate a behavioral method for separating reward from motor/performance effects [8].

Other studies involving picrotoxin [25, 29, 38] employed reward threshold or other measures which are much more reward-specific, although one study [38] used the two-lever autotitration technique which has received some criticism [16], and another study [29] delivered picrotoxin via accumbens microinjection as opposed to systemically. However, the basic question remains as to why GABA agonists and antagonists should both reduce reward. Related to this question is the finding that benzodiazepines, which act at the GABA receptor complex [35], tend to increase self-stimulation [9,11] possibly through a reduction in stimulation-produced aversive effects that are often mixed with the primary reward effect [16]. Further research using reward-specific behavioral methods is required to answer the question of why GABA agonists and antagonists could produce the same effects on self-stimulation reward.

Finally, we observed sedative-like effects of $LG_2$ at high doses, as would be expected [12, 14, 18, 26, 28, 35], but these effects were not sufficient to impair self-stimulation behavior. For example, some animals were observed to lie on the cage floor on their sides with eyes closed under $LG_2$. However, when the beginning of the next rate-frequency test was signaled by the illumination of the house light, casual observation and the form of the data collected suggest that all rats quickly righted themselves and pressed the lever vigorously. Thus, this motor suppression effect was not obligatory. Certainly, fewer statistical effects were seen on motor/performance capacity compared to reward function (Table 2 vs. 3).

In conclusion, the total results suggest that $LG_2$ is a biologically active compound with the capacity to alter affective/limbic function, e.g., reward, without significant effects on operant motor/performance, which is remarkable considering the diffuse distribution of GABA in the brain.

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