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## Effects of anxiolytic drugs on rewarding and aversive behaviors induced by intracranial stimulation.

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## Abstract

In considering the characteristics of the action of anxiolytic drugs and their mechanism in the brain, it may be necessary not only to study the behavioral pharmacology but also to perform brain site research. In the present study, the action of anxiolytic drugs was examined with respect to various behaviors that were induced by stimulating the brain areas related to emotions such as reward (pleasure) or aversion in rats. First, the low rate of response in lateral hypothalamic self-stimulation behavior was induced by schedules of low current brain stimulation, variable interval (VI) and differential reinforcement of low rate (DRL). Anxiolytic drugs such as benzodiazepines facilitated these low-rate responses. The drug susceptibility was highest in the low current stimulation, lower in the VI stimulation, and lowest in the DRL stimulation schedules. Furthermore, it was found by the auto-titration method in intracranial self-stimulation behavior that anxiolytic drugs decreased the threshold of stimulation reward. Second, it was recognized using the decremental lever pressing (DLP) paradigm that anxiolytic drugs increased the threshold of aversive stimulation of mesencephalic dorsal central gray (DCG), and this increasing effect of the drug was antagonized by GABA receptor blockers such as bicuculline. Finally, it was examined whether or not the conflict situation is established by combining brain stimulation reward and aversion, such as foot-shock or DCG stimulation. As a result, the conflict behavior was established by combining not only the brain stimulation reward and foot-shock aversion, but also the brain stimulation reward and DCG stimulation aversion. Further anxiolytic drugs exhibited anti-conflict action in both situations. The susceptibility of anxiolytic drugs was higher with respect to the conflict behavior induced by intracranial reward and aversion than to that induced by the conventional method based on milk reward and foot-shock aversion. These results suggest that behavioral methods using brain stimulation can examine the mechanisms of direct drug action at the brain stimulation site. Indeed, in the present brain stimulation behavioral study, anxiolytic drugs such as benzodiazepines increased the stimulation threshold in lateral hypothalamic self-stimulation and inhibited the DCG aversive stimulation, thus resulting in an anticonflict action of the drugs.

**KEYWORDS:** anxiolytic drugs, lateral hypothalamic self-stimulation, escape behavior induced by mesencephalic dorsal central gray stimulation, conflict behavior, rats

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*Review*

## Effects of Anxiolytic Drugs on Rewarding and Aversive Behaviors Induced by Intracranial Stimulation

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In considering the characteristics of the action of anxiolytic drugs and their mechanism in the brain, it may be necessary not only to study the behavioral pharmacology but also to perform brain site research. In the present study, the action of anxiolytic drugs was examined with respect to various behaviors that were induced by stimulating the brain areas related to emotions such as reward (pleasure) or aversion in rats. First, the low rate of response in lateral hypothalamic self-stimulation behavior was induced by schedules of low current brain stimulation, variable interval (VI) and differential reinforcement of low rate (DRL). Anxiolytic drugs such as benzodiazepines facilitated these low-rate responses. The drug susceptibility was highest in the low current stimulation, lower in the VI stimulation, and lowest in the DRL stimulation schedules. Furthermore, it was found by the auto-titration method in intracranial self-stimulation behavior that anxiolytic drugs decreased the threshold of stimulation reward. Second, it was recognized using the decremental lever pressing (DLP) paradigm that anxiolytic drugs increased the threshold of aversive stimulation of mesencephalic dorsal central gray (DCG), and this increasing effect of the drug was antagonized by GABA receptor blockers such as bicuculline. Finally, it was examined whether or not the conflict situation is established by combining brain stimulation reward and aversion, such as foot-shock or DCG stimulation. As a result, the conflict behavior was established by combining not only the brain stimulation reward and foot-shock aversion, but also the brain stimulation reward and DCG stimulation aversion. Further anxiolytic drugs exhibited anti-conflict action in both situations. The susceptibility of anxiolytic drugs was higher with respect to the conflict behavior induced by intracranial reward and aversion than to that induced by the conventional method based on milk reward and foot-shock aversion. These results suggest that behavioral methods using brain stimulation can examine the mechanisms of direct drug action at the brain stimulation site. Indeed, in the present brain stimulation behavioral study, anxiolytic drugs such as benzodiazepines increased the stimulation threshold in lateral hypothalamic self-stimulation and inhibited the DCG aversive stimulation, thus resulting in an anticonflict action of the drugs.

**Key words:** anxiolytic drugs, lateral hypothalamic self-stimulation, escape behavior induced by mesencephalic dorsal central gray stimulation, conflict behavior, rats

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## I. Introduction

To measure the anti-anxiety activity of anxiolytic drugs in animals, various emotional situations that can be induced using learning behaviors have often been utilized. In these situations, “conflicts” of behavior are induced by combinations of rewards, such as milk or food, and aversions, such as foot-shock [1], and “low-rate responses” which involve lever pressing that provide a reward of food or milk or which involve escaping or avoiding the aversive foot-shock are induced [2, 3].

When an animal behaves in a normal manner, there is always a “drive” inducing that behavior. The behavioral pattern is attributed to the kinds of “drives” that are operative. That is, if the subject requires a stimulation, which is the drive, it is considered to be a positive “drive”, *i.e.* following the lever pressing the behavior is rewarded in the Skinner box. If the subject avoids or escapes the stimulation, it is considered to be a negative “drive”, *i.e.* aversive behavior such as avoidance or escape behaviors. The “conflict” situation mentioned above is induced by the combination of positive (food or milk reward) and negative “drives” (foot-shock aversion). Anxiolytic drugs have a repairing action in this situation known as an anti-conflict action [4-6]. However, this action creates certain problems for assessing the activity of anti-anxiety drugs, because the maintenance of healthy conditions in animals is somewhat changed and gastrointestinal drug absorption may be altered in such a deprived state.

However, various emotional situations can be induced by brain stimulation. For example, the medial forebrain bundle (MFB) was reported to be a site activated during rewarded behavior. That is, electrical stimulation of this site causes self-stimulation behavior [7]. Other sites are activated during aversive behavior, such as the periventricular system including the mesencephalic dorsal central gray (DCG) matter. That is, brain stimulation at the DCG induces escape or avoidance behavior [8, 9]. The “conflict” situation can also be established by combining brain reward stimulation with aversion to foot-shock or brain aversive stimulation. In these experimental situations, there is no need to consider experimental conditions such as a long-term deprived state.

In the present study, the effects of anxiolytic drugs on various emotional situations induced by learning behavior based on brain rewarding or aversive stimulation are discussed with respect to the experimental techniques and

the mechanisms of drug action.

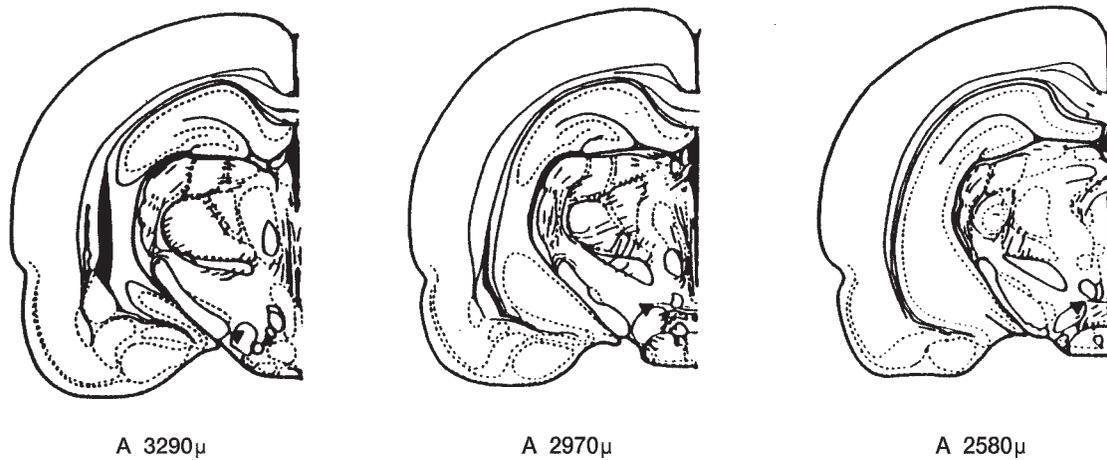
## II. Methods for Brain Stimulation Reward and Aversion

### II-1 Implantation of Electrode for Brain Stimulation

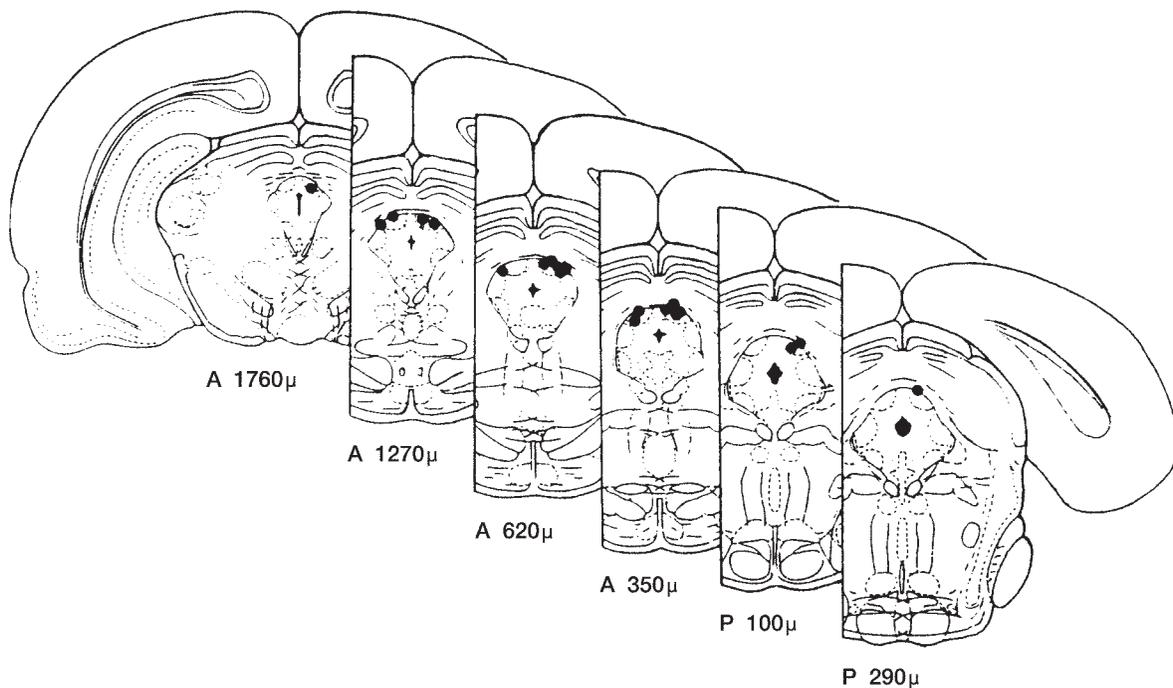
A chronic electrode was implanted at a specific brain site in a rat, and current flowed to that brain site from the electrode. The rat could either elicit the brain stimulation by pressing a lever in a Skinner box (“self-stimulation behavior”) or could stop the brain stimulation by pressing a lever in a Skinner box (“escape behavior”) [7, 10]. The MFB in the lateral hypothalamus and tegmentum mesencephali is known as a representative brain rewarding site [11] (Fig. 1), and the mesencephalic DCG of the periventricular system is a brain aversive site [12, 13] (Fig. 2).

The method for implanting the chronic electrode in the brain [14] was described previously. Male Wistar rats were anesthetized with 40 mg/kg pentobarbital-Na, *i.p.* and stereotaxically and chronically implanted with bipolar stainless steel electrodes in the lateral hypothalamus at either the MFB for intracranial self-stimulation reward, or in the mesencephalic DCG of the periventricular system for brain stimulation aversion, according to de Groot’s coordinates [15], König & Klippel [16], or Paxinos & Watson [17]. The electrode consisted of 2 stainless steel wires which were 250  $\mu\text{m}$  in diameter and were insulated except for 0.5 mm at the electrode tip. The distance between both poles was 0.5 mm. The electrodes were bilaterally inserted into the target sites at a vertical angle for self-stimulating animals, and at a 15° angle to avoid piercing the sagittal venous sinus. After inserting the electrode in the MFB or DCG, it was fixed with dental cement to the skull and carefully connected with solder to the foot of the connector-socket. The connector-socket was also fixed in the cement with 2 screw-nails in the skull. All animals were administered approximately 100,000 units of Penicillin G in the hip muscle for 2-3 days after the surgery. Training to press a lever in a Skinner box for intracranial self-stimulation or escape was carried out about 10 days after the implantation surgery.

At the end of the experiment, all of the animals were administered an overdose of pentobarbital-Na. The head was intra-cardially perfused with 0.9% saline and 10% formalin. The brain was cut into 40  $\mu\text{m}$  sections using a freezing microtome, followed by staining with



**Fig. 1** Localization of electrode tip (triangles) in the medial forebrain bundle for self-stimulation. The lower numbers indicate the anterior (A) distance from the zero plane according to König and Klippel's atlas [16].



**Fig. 2** Localizations of electrode tips in the dorsal central gray area for aversive stimulation. The lower numbers indicate the anterior (A) and posterior (P) distances from the zero plane according to König and Klippel's atlas [16].

cresylviolet. The localization of the implanted electrode tips in the MFB or DCG was verified by inspection of the stained sections.

## II-2 Training for Brain Stimulation Reward or Aversion

At least 10 days were allowed for recovery before commencing the training for intracranial self-stimulation. The experiments were carried out using a Skinner box.

Each animal was placed in the box, and a stimulating cable was connected to the electrode plug mounted on the animal's head. A lever press activated a counter and resulted in brain stimulation.

Each animal was trained to press a lever for the brain stimulation reward, which was obtainable on a continuous reinforcement (crf) schedule. Each brain stimulation reward consisted of a 60 Hz sinusoidal current lasting for 0.2 sec, and was individually adjusted for each rat. The stimulation current was gradually increased until the animal began to respond at a heightened activity level. Five to 10 daily training sessions (15 min/day) were given to each animal. The stimulation current intensity was determined to be the approximate level that maintained the maximum response rate (high rate) of intracranial self-stimulation without producing gross motor disturbances or convulsion.

To observe these behaviors, operant conditioning control equipment and cumulative recording equipment in addition to the Skinner box (25 cm wide, 30.8 cm depth, 27.7 cm height) was required. The cumulative recording equipment could operate at the output from the operant conditioning control equipment.

The escape behavior was measured in the DCG stimulation [13, 18] as follows. Using the chronic electrode implanting method, the rat brain was fixed in the stereotaxis apparatus, and a bipolar electrode was implanted in the DCG. At that time, the electrode was inserted at a 15° angle from the vertical surface to avoid blood vessel hemorrhage on the center line. Between one and two weeks after the surgery, lever pressing to escape the DCG stimulation was practiced with the animal exhibiting a running and rapid movement. That is to say, a sound at 1,850 Hz was administered for 5 sec (warning period), and immediately afterward the DCG was stimulated electrically (stimulation period). When the animal pressed a lever in the Skinner box during the warning period it was judged as avoidance behavior, and when it pressed a lever during the stimulation period it was judged as escape behavior. Pressing the lever turned off the brain stimulation. A swivel joint was mounted in the ceiling of the chamber holding the electrode lead, which allowed the animal to move freely.

The drugs used as anxiolytics were benzodiazepines, including diazepam, chlordiazepoxide and bromazepam *etc.* All drugs were suspended in 0.5% carboxymethylcellulose-Na (CMC) solution and were orally administered. As a control, 0.5% CMC was also

administered. In each test, we recorded the animal's lever-pressing response before and after drug administration. At least 2 weeks elapsed between each drug administration.

### III. Brain Stimulation Behavior and Action of Anxiolytic Drugs

In general, when examining the anti-anxiety action of drugs in rats, methods that may induce a low-rate response of lever pressing, schedules of variable interval (VI) and differential reinforcement of low rate (DRL) based on food & milk intake, and foot-shock aversion [2, 3] were utilized. The anti-anxiety drug facilitates these behaviors. However, when setting the situation, the animal has to be exposed to hunger and/or thirst for long periods. In these situations, the action mode of the drugs may change.

However, brain stimulation can cause various syndromes like self-stimulation reward or escape aversion according to differences in the stimulation sites. In this section (III), the intracranial self-stimulation induced by the MFB stimulation, the escape behavior induced by stimulation of a dorsal part of the mesencephalic central gray matter (DCG) and the effect of anti-anxiety drugs on these behaviors are discussed.

#### III-1 Brain Stimulation Reward and Drug Action

##### III-1-A Low-Rate Response and Drug Action

*i) Low-rate Response Induced by Low Current Stimulation.* The animal pressed the lever in the Skinner box when the electrical stimulation was administered to the site of the implanted brain electrode. The lever-pressing response rate was different for low and high stimulation currents. After the response to the intracranial self-stimulation reached a high rate under the crf-schedule and became stable over three successive days, the current intensities were gradually reduced until the responses were decreased to below half of the high-rate responses. The anxiolytic drugs were administered when the responses were stabilized with 10% variation through 2 successive days [14].

In cases with high response rates, the inhibitory action of the drug was easily recognized, but in cases with low response rates, not only the inhibitory action but also the facilitating action of the drugs was observed. Anti-anxiety drugs at low doses markedly facilitated the low-

rate response induced by low current brain stimulation (Fig. 3). For example, it was recognized in the present study that the low-rate response of hypothalamic self-stimulation behavior induced by low current brain stimulation was markedly increased by diazepam at doses ranging from 1 to 10 mg/kg, p.o., but was suppressed at 80 mg/kg p.o. However, the high-rate response was reduced by diazepam at 180 mg/kg p.o. Triazolam, one of the benzodiazepines, also facilitated the low-rate response at doses ranging from 2 to 40 mg/kg, p.o., but suppressed it at doses greater than 80 mg/kg p.o. The high-rate response was unaffected even at doses of 40–180 mg/kg, p.o. Furthermore, the facilitating effects of this response were also recognized during amphetamine and morphine administration.

**ii) Low-Rate Response Induced by VI Schedule.** The low-rate response was also possible in the intracranial self-stimulation [14, 19]. After the

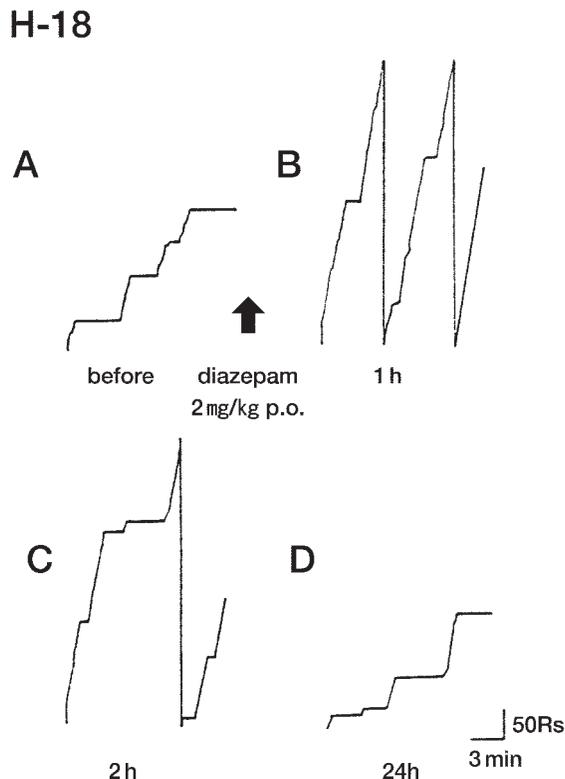
animals were trained to perform lever pressing at a high rate under the crf schedule, a low-rate of lever pressing could be obtained in the VI schedule (if an animal presses a lever with some variable interval, the animal can obtain an efficient target of brain stimulation rewards), and the drug action can be examined. Anti-anxiety drugs facilitated a low-rate response in the VI schedule.

It was recognized in the present study that the low-rate response induced by the VI schedule was dose dependently increased by chlordiazepoxide at doses ranging from 5–10 mg/kg, p.o. and by diazepam at doses ranging from 2–10 mg/kg, p.o.

**iii) Low-Rate Response Induced by DRL Schedule.** Animals that exhibited a high-rate response of self-stimulation were shifted to the DRL schedule (the rewarding stimulation was efficiently obtained when the lever was pressed at a predefined interval), thus resulting in a low-rate response by adjusting the time of the DRL schedule. In the DRL schedule, which is based on food and milk rewards, the DRL schedule at 20 and 30 sec is often utilized [3]. Using this DRL schedule with intracranial self-stimulation, it is possible to obtain a stabilized low-rate response with the DRL at 8 sec [19]. The drug action was examined when a daily change in the number of all lever-pressing responses was within  $\pm 10\%$  and was maintained for 2 days.

Diazepam (5 and 10 mg/kg) and meprobamate (200 mg/kg) caused significant increases in the response rates during the first 5 min of a session, but not thereafter. Bromazepam (1 and 5 mg/kg) also caused a significant increase in the rates during the first and second 5 min. However, chlorpromazine (20 mg/kg) had no effect in the first 5 min but caused decreases in the second and third 5 min periods. The advantage of the brain stimulation reward over the food reward is that the possible effects of the drugs on hunger motivation need not be considered.

**iv) Characteristics of the Low-Rate Response Induced by Various Kinds of Schedules in Intracranial Self-stimulation.** Table 1 shows the characteristics of the low-rate responses induced by various kinds of schedules in intracranial self-stimulation. In the low-current stimulation study, the response rate was current-dependent. However, the lever-pressing response, which stopped temporarily, was sometimes observed during low-current brain stimulation. Accordingly, it was not easy to obtain a stable lever-pressing response in a low-current stimula-



**Fig. 3** Effect of diazepam on the low-rate response induced by the low-current hypothalamic stimulation. Cumulative recordings were made when diazepam was administered orally at a dose of 2 mg/kg. A, before administration; B, C and D, 1 h, 2 h and 24 h after administration, respectively.

Table I Characteristics of various kinds of low rate responding behaviors in intracranial self-stimulation

Schedules	Low current	VI	DRL-1 sec	DRL-8 sec
Stability of response rate	Unstable	Most stable	Most stable	Stable
Current dependency	+++	++	++	+
Sensitivity to benzodiazepines	+++	++	++	+

+, Slight; ++, Marked; +++, Extreme; DCG, Mesencephalic dorsal central gray; DRL, Differential reinforcement of low rate; VI, Variable interval.

tion, although the sensitivity to anti-anxiety drugs was high. A time period of between 20 and 40 days was required to obtain a stable response.

However, we could also obtain a low-rate response under the VI schedule in addition to the FR schedule. Under these schedules, problems were noted during the stable responses and in the time necessary to obtain a stable response. With regard to the VI schedule, a stable response could be obtained without temporarily stopping the lever pressing as in the low-current stimulation. In addition, it was possible to take only 5-7 days to reach stability; this interval was very short in comparison with those in the low-current stimulation and FR schedules.

As mentioned above, the low-rate responses induced by low-current stimulation and the FR schedule were unstable, but those induced by VI and the DRL schedule were very stable. Furthermore, less time was required to reach a level of stability from which to investigate anti-anxiety drugs in the VI and DRL schedules.

In the low-current stimulation, the degree of current dependency was in the order VI > DRL, and this result was also related to the stability. The stability of the lever-pressing response and the ease with what a stability was reached for setting the situation ranked in the order DRL > VI > low current stimulation. However, the anti-anxiety drugs facilitated a low-rate response of these schedules. The drug susceptibility ranked as follows: DRL < VI < low-current stimulation.

### III-1-B Auto-titration Using Two-Lever Pressing and Drug Action

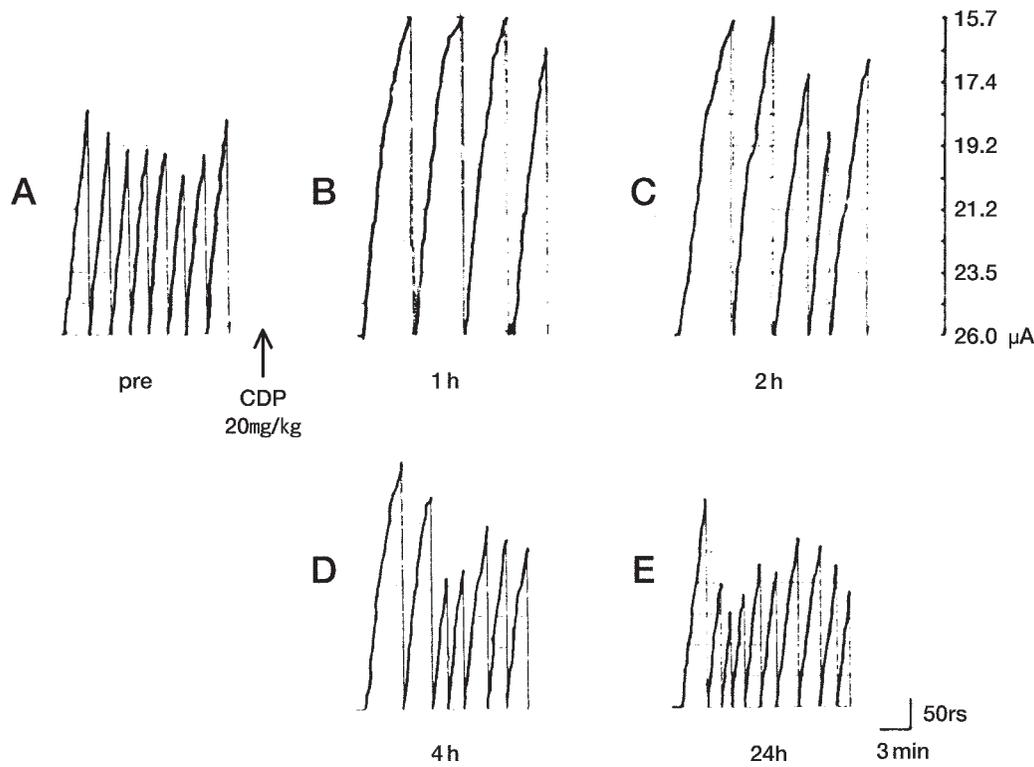
As mentioned above, several studies reported that response rates of intracranial self-stimulation are facilitated by anti-anxiety drugs. Some of these studies suggested that anti-anxiety drugs may enhance the susceptibility to brain stimulation rewards. However, no direct evidence has been provided. That is to say, it is unclear whether such a facilitation is due to a change in the rewarding

effect of brain stimulation itself and/or due to an alteration in motor activity, arousal level, *etc.* The influence of anti-anxiety drugs on the intracranial self-stimulation threshold while facilitating the response of this self-stimulation behavior requires clarification.

It is possible to examine the drug action in order to detect the threshold of self-stimulation using the auto-titration technique [20-22]. The present auto-titration technique is similar to that reported by Schaefer *et al.* [20], but with several modifications. The delivered negative square wave pulses at a frequency of 100 pulses/sec with a duration of 0.2 sec correspond with each pressing of the brain stimulation lever. After achieving a stable reset response in reset training, the animal was exposed to the auto-titration procedure. This procedure consisted of conventional intracranial self-stimulation, except the brain stimulation current intensity was decreased by 2% after 20 lever presses for self-stimulation, and the animal could press the other reset lever at any time to reset the stimulation. The stimulation current when the animal presses the reset lever is referred to as the "reset current." In this technique, to automatically lower the brain stimulation, an electro-controlling stimulator, a Skinner box and a cumulative recorder are necessary to achieve the step-down of the brain stimulation threshold.

First, the self-stimulation was practiced until the forebrain stimulation responses under the crf-schedule reached a specific criterion (more than 1,000 responses/15 min), using one side lever in the Skinner box. During this training, the intensity of the brain stimulation currents dropped 100% to zero at the 50th press and returned to the initial intensity when the animal pressed the reset lever. So, using the auto-titration technique mentioned above, the threshold of stimulation reward when the drug is administered can be learned.

In Fig. 4, the representative cumulative curve when an anti-anxiety drug was administered is shown. The



**Fig. 4** Effect of chlordiazepoxide (CDP) on the stimulation threshold in auto-titration behavior. Representative cumulative recordings were made when CDP, at a dose of 20 mg/kg, was administered orally. **A**, Before administration; **B**, **C**, **D** and **E**, 1 h, 2 h, 4 h and 24 h after administration, respectively. Stimulation currents are indicated on the right side.

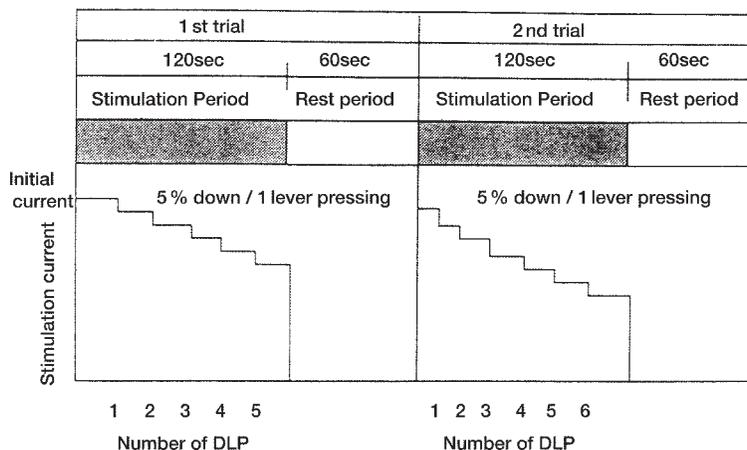
reset currents were significantly lowered by chlordiazepoxide (5.0–20 mg/kg) accompanied by a significant increase in self-stimulation. However, diazepam (1.0 mg/kg) did not lower the reset current, although it induced a significant increase in self-stimulation. Meprobamate (100 mg/kg) also lowered the reset current with an accompanied increase in self-stimulation. From these findings, increased susceptibility to the brain stimulation current may be involved in the facilitating effects of anti-anxiety drugs in self-stimulation.

### III-1-C Possible Explanation for the Facilitatory Action of Antianxiety Drugs on Self-stimulation

As mentioned above, the antianxiety drugs such as benzodiazepines not only facilitated the reward responses in various kinds of self-stimulation behaviors, such as low-rate responses induced by low-current stimulation, VI and DRL schedules [8, 14, 19], but also decreased the stimulation threshold [22].

On the other hand, it is well known that benzodiazepines enhance the function of the GABAergic synaptic mechanism in the brain, which secondarily causes reduction of the serotonergic activity [23], and also that the dopaminergic mechanism is related to the appearance of the intracranial self-stimulation behavior [11, 24–26], suggesting that drugs such as benzodiazepines enhance the GABAergic nerve function, and modulate the mesocortico-limbic dopaminergic system. In addition, Borisenko *et al.* [27] observed that 1-(3-chlorophenyl) piperazine, a 5-HT agonist, inhibited the self-stimulation, and ketanserin, a 5-HT<sub>2A</sub> antagonist, increased the self-stimulation, *i.e.*, indicating the relation of serotonergic inhibitory function on self-stimulation behavior.

From these findings, it may be suggested that the facilitation of self-stimulation by antianxiety drugs may be attributed to the increase of dopamine caused by the reduction of serotonergic nerve function via the GABAergic mechanism of antianxiety drugs. The GABAergic mechanism may be directly related to dopaminergic nerve



**Fig. 5** Schedule for the decremental lever pressing (DLP) paradigm. The animal began to press a lever to stop or decrease the DCG stimulation; the value of the stimulation decreased by 5% at each lever press. It was possible to measure the stimulation current as the current threshold which did not react to the aversion.

function in self-stimulation, although it is not possible to neglect the possible involvement of other mechanisms.

### III-2 Brain Stimulation Aversion and Drug Action

#### III-2-A Effects of anti-anxiety drugs on aversive behavior induced by DCG stimulation

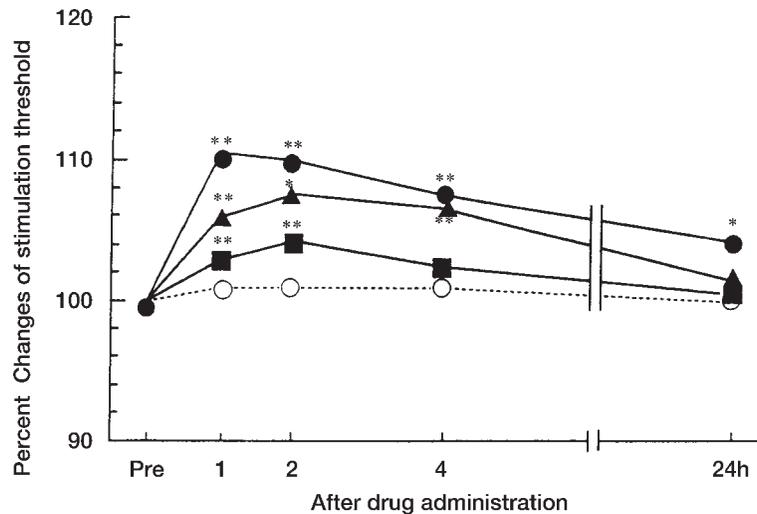
Electrical stimulation of a DCG provokes aversive symptoms such as running, jumping and escape response [6, 9, 28, 29]. Animals that exhibit the above symptoms can learn to press a lever to stop the aversive DCG stimulation in the Skinner box (operant escape response). The brain site that causes escape behavior after electrical stimulation in the brain is a region that belongs to the ventricular cerebral system, and includes the ventromedial hypothalamus, tegmentum mesencephali and mesencephalon central gray area [9]. It has been suggested that this aversion to brain stimulation might serve as a model of anxiety. Indeed, the escape behavior induced by DCG stimulation is suppressed by anxiolytic drugs such as benzodiazepines [30]. After establishing lever pressing to escape aversive DCG stimulation, the training of the decremental lever-pressing paradigm in rats that acquired the escape behavior via the lever pressing (DLP) was carried out (Fig. 5). The animal in training began to press a lever to stop or decrease the DCG-stimulation, with the value of the DCG stimulation decreasing by 5% at each lever press. It was possible to measure the stimulation current as the current threshold

that did not react to the aversion. That is to say, a decrease in the lever-pressing responses may inversely show a rise in the threshold of aversive DCG stimulation. The number of lever presses reached 4–6 times/trial as a result of the stimulation current being varied. Ten trials/day (for 30 min) were practiced, and the drug action was examined in rats in which the lever pressing was stabilized.

In this behavior, we observed that anti-anxiety drugs such as benzodiazepines increased the DCG stimulation threshold (Fig. 6) [31]. The drug action strength, *i.e.* the strength of the anti-conflict action of the drugs, were ranked as follows: bromazepam > diazepam > chlordiazepoxide. In addition, the increased action of benzodiazepines on the stimulation threshold was antagonized by bicuculline, a GABA receptor blocker [31], while muscimol, a GABA receptor agonist, increased the DCG-stimulation threshold.

#### III-2-B Possible Explanation for the Inhibitory Action of Anti-anxiety Drugs on DCG Stimulation Behavior

In the present study, it was shown that anti-anxiety drugs rise to the threshold of aversive stimulation of DCG, thus suggesting an inhibitory action of the drug [31]. We also previously reported that not only the cholinergic mechanism but also the serotonergic mechanism are related to this aversive behavior [29]. Further, Castilho *et al.* [32] reported that the aversive behavior



**Fig. 6** Effects of chlordiazepoxide (CDP) on the DCG stimulation threshold. CDP at doses of 5 (■), 10 (▲) and 20 (●) mg/kg were orally administered. The vehicle (○) corresponding to each drug was orally administered to respective control animals. Asterisks indicate significant differences from the vehicle control group. \*  $P < 0.05$ , \*\* $P < 0.01$ .

elicited by aversive stimulation of the inferior colliculus (a part of the periventricular system) was inhibited not only by benzodiazepine (medazepam), but also by methysergide, a nonspecific antagonist of 5HT, and ketanserine, a specific antagonist of a 5HT<sub>2</sub>. Brandao *et al.* [33] observed that dorsal periaqueductal gray (DPAG), a deep layer of the superior and an inferior colliculus, induces aversive responses such as escape behavior.

On the other hand, it is also believed that GABA receptors may be related to escape behavior induced by brain stimulation. Bovier *et al.* [34] reported that sodium valproate and progabide, which are pro-GABAergic drugs, increased the latency of the escape behavior induced by DCG stimulation. The present authors have also shown that the DCG-stimulation threshold was increased by muscimol, a GABAergic agonist, and was decreased by bicuculline and picrotoxin, and the combined increasing effect of diazepam and GABA agonists in the DCG stimulation threshold was also antagonized by bicuculline and picrotoxin, suggesting that the suppression of escape behavior by benzodiazepines may be attenuated via a GABAergic mechanism [6, 31]. That is to say, GABA, 5HT, and ACh-related mechanisms may be concerned with the appearance of escape behavior induced by DCG stimulation.

Based on these findings, the action of anti-anxiety drugs on DCG stimulation-induced aversion may be

attributed to the mechanism of GABAergic and serotonergic nerves [31, 35].

#### IV. Conflict Situation Based on Intracranial Reward Stimulation and Aversion

The conflict situation induced by combining food or milk rewards with foot-shock aversion in rats has been documented to evaluate the anti-conflict action of anti-anxiety drugs [1]. The anti-anxiety drugs showed antagonism for such situations, a finding which is in line with the clinical anxiolytic effect on behavioral suppression induced by various aversions [5, 6]. Based on intracranial self-stimulation reward, the conflict situation may be established by combining aversions with foot-shock or intracranial DCG stimulation. These techniques, however, have been used under long-term hunger conditions which create certain problems when assessing the activity of anti-anxiety drugs. Namely, the maintenance of a healthy condition in the animals is somewhat difficult, and gastrointestinal drug absorption may be altered in such a deprived state. Therefore, in this section, the conflict situation induced by combining the intracranial reward and aversion of foot-shock or mesencephalic stimulation is described.

#### IV-1 Conflict Situation Induced by Combination of Intracranial Reward Stimulation and Foot-Shock

After recovering from the implantation surgery, each animal was placed in a Skinner box, and the stimulating cable was connected to the electrode plug mounted on the head. The stimulation currents were gradually increased until the animal began to show self-stimulation behavior, accompanied with sniffing and a heightened activity level. This training was performed using a crf-schedule. After the lever pressing for self-stimulation on a crf-schedule reached the maximum response rate, the reinforcement contingency was gradually altered until all rats performed a stable response for self-stimulation under the FR-5 schedule without decreasing the maximum lever press rate. Thereafter, foot-shock punishment (0.1–1.5 mA, 0.2 sec in duration) was combined with intracranial self-stimulation. The self-stimulation reward, foot-shock punishment procedure was similar to the method of Geller and Seifter [1], except that the previous researchers used a food reward. The test consisted of 2 (15 min) sessions, in which a 12-min period without punishment was followed by a 3-min period with punishment. The rat responded to a brain stimulation reward under the FR-5 schedule during the non-punishment period. The punishment period was accompanied by a tone of 1,850 Hz and a cure light near the lever, and each response during this period was rewarded with brain stimulation and was concurrently punished with a brief electric foot shock. The intensity of the foot shock was gradually increased and adjusted in each animal until the response rate during the punishment period was suppressed to less than 10 responses/3 min, while the unpunished response under the FR 5 schedule remained at relatively high levels. Based on the self-stimulation behavior of the rat in a Skinner box, a conflict situation was established by combining foot-shock punishment with brain self-stimulation [36].

The schedule during the punishment period was similar to that used by Geller and Seifter [1]. In this conflict situation based on self-stimulation behavior, both diazepam and bromazepam, at doses greater than 10 mg/kg, p.o., caused a marked increase in the lever-pressing response during the period with punishment (Fig. 7). The effective dose of diazepam for attenuating the suppression of behavior during the punishment period with this self-stimulation reward was slightly lower in comparison with the findings of our previous study using a food

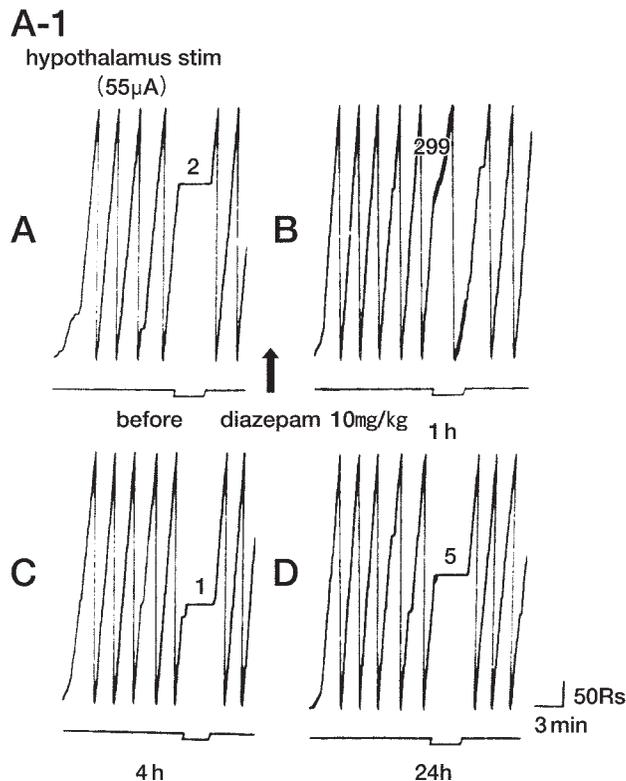


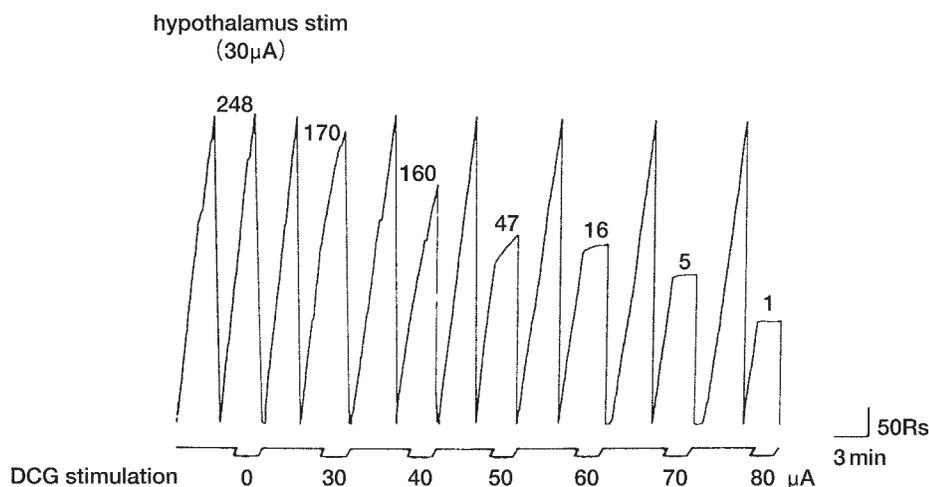
Fig. 7 Effect of diazepam on a "conflict" situation in lateral hypothalamic self-stimulation behavior. Cumulative recording of the lever pressing before A and 1 h B, 4 h C and 24 h D after administration of diazepam 10 mg/kg, p.o. The punishment period (3 min) is indicated on the lever line in each panel. The number of lever presses during the punishment period is included in the figure.

reward [8].

#### IV-2 Conflict Situation Induced by Combination of Intracranial Reward Stimulation and Brain DCG Aversive Stimulation

As mentioned above, there are 2 brain areas in which brain stimulation drives the reward response and the aversive response. The former refers to the stimulation reward for self-stimulation and the latter signifies the impulse to escape the brain stimulation. Based on self-stimulation of the rat in the Skinner box, a conflict situation was established by combining foot-shock punishment with brain stimulation [36]. In addition, the conflict situation was established by combining this self-stimulation behavior with the MFB stimulation and aversive escape behavior with DCG stimulation [29].

Fig. 8 shows the effect of contingent DCG stimula-



**Fig. 8** Behavioral suppression ("conflict" situation) obtained by combining hypothalamic self-stimulation reward and dorsal central gray area stimulation aversion. Ordinate, lever-pressing responses; Abscissa: time. The punishment period (3 min) is indicated on the lower line. The number in the cumulative record indicates the responses for lever pressing during the punishment period.

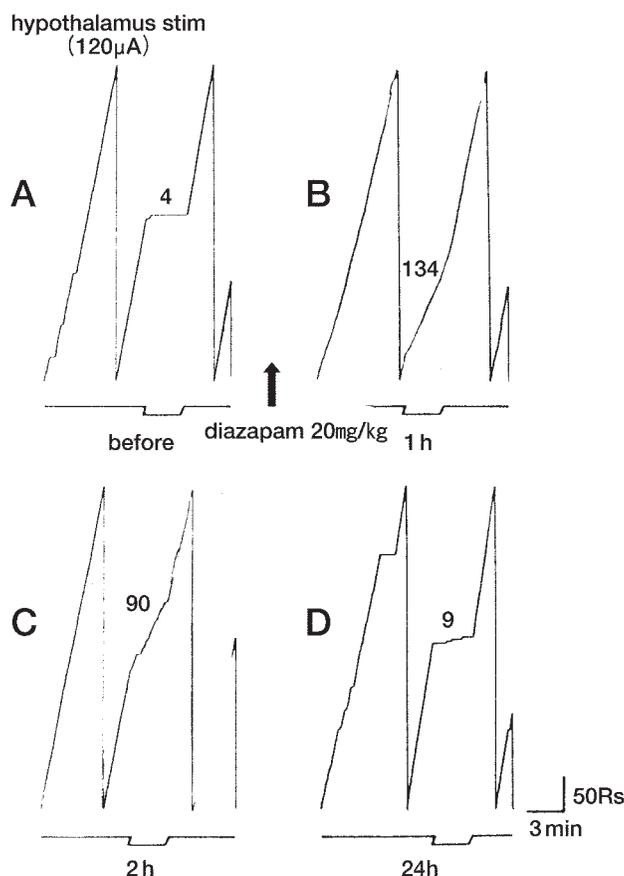
tion on lever pressing that is maintained by a rewarding electrical stimulation of the lateral hypothalamus. Namely, the lever pressing for self-stimulation was suppressed with the rise in the DCG stimulation current, thus establishing the conflict situation. In Fig. 9, the anti-conflict effect of benzodiazepines on this behavior is shown.

The effective doses of benzodiazepines in the present situation using lateral hypothalamic self-stimulation reward and DCG stimulation aversion were markedly lower than in situations involving conventional reinforcements such as food and milk rewards [30]. Furthermore, the drug effects in the present situation were more sensitive in comparison with those in the situation using intracranial self-stimulation reward and foot shock [36]. Benzodiazepines were suggested to act not by increasing the appetite motivation leading to behavior, but rather by releasing behavioral suppression, *i.e.* by disinhibitory action [5].

### V. General Discussion of the Action of Antianxiety Drugs on Conflict Behaviors Induced by Combining Self-Stimulation Reward with Foot-Shock or Brain Stimulation Aversions

In the present study, the actions of anxiolytic drugs on various behaviors induced by stimulating the brain sites related to the expression of reward or aversion were discussed. The characteristics of the drug actions are summarized in Table 2.

In the intracranial self-stimulation behavior, anxiolytic drugs such as benzodiazepines facilitated the low-rate



**Fig. 9** Effect of diazepam on a "conflict" situation induced by combining hypothalamic self-stimulation reward with dorsal central gray area stimulation aversion. Cumulative recording of the lever pressing was done before (A), 1 h (B), 2 h (C) and 24 h (D) after administration of diazepam 20 mg/kg PO. The punishment period (3 min) is indicated in the lower line of each panel.

**Table 2** Characteristics of the action of anxiolytic drugs on various kinds of behaviors induced by intracranial stimulation reward and/or aversion

Behaviors	Action of anxiolytic drugs
Hypothalamic self-stimulation behaviors	
Low-rate responding (LC, VI, DRL schedules)	Increase (Susceptibility; LC > VI > DRL)
Threshold of stimulation reward (Auto-titration schedule)	Decrease
Escape behavior to DCG stimulation aversion	
Threshold of stimulation reward (Decremental lever-pressing paradigm)	Decrease
Conflict behaviors	
Responding based on self-stimulation reward and foot-shock aversion	High susceptibility
Responding based on self-stimulation reward and DCG stimulation aversion	Higher susceptibility

DCG, Mesencephalic dorsal central gray; DRL, Differential reinforcement of low rate; LC, Low current stimulation; VI, Variable interval.

response induced by the low current stimulation, VI and DRL schedules. The drug susceptibility of the three schedules was ranked as follows: low current stimulation > VI > DRL. Furthermore, anxiolytic drugs decreased the threshold of the self-stimulation reward. However, with respect to the escape behavior seen after aversive DCG stimulation, anxiolytic drugs increased the threshold of the brain stimulation aversion. Furthermore, the conflict situations were established by combining intracranial stimulation reward with foot-shock or DCG stimulation aversion, and the anti-conflict actions of anti-anxiety drugs were recognized as similar to conventional conflict behavior. These behaviors induced by brain stimulation may be useful for clarifying the mechanism of action of the central nerve-acting drugs.

Benzodiazepines increase the low-rate lever pressing maintained by DRL or VI procedures with food rewards [37, 38] or brain stimulation rewards [8, 19]. The effective doses of benzodiazepines in the present situation using the self-stimulation reward and DCG stimulation aversion were markedly lower than in situations involving the DRL response, or in conflict situations involving conventional reinforcements such as food and milk [30]. Further, the drug effects in this situation were more sensitive in comparison with conventional rewards and foot-shock stimulations [36].

It is also recognized that benzodiazepines facilitate

GABAergic synaptic mechanisms, which secondarily cause a reduction in serotonergic activity [21]. Furthermore, Zarevices & Setler [39] showed, using a 2-lever intracranial self-stimulation paradigm, that increased reward, as indicated by a lower reward threshold, was produced by the GABAergic agonist, muscimol. Further, 5HT agonist depressed the self-stimulation, and ketamine 5HT<sub>2A</sub> antagonist facilitated the self-stimulation; it was shown that self-stimulation was caused by a reduction of the serotonergic function [27].

However, it was shown that benzodiazepines exhibit an inhibitory action on brain stimulation aversion; *i.e.*, they increase the stimulation threshold of the brain aversive stimulation [31]. It was suggested that 5HT reduced the aversive neural mechanism associated with the DCG area [28], and the increases in the DCG-stimulation threshold induced by benzodiazepines were antagonized by bicuculline and picrotoxin, GABA receptor blockers [31]. Further, Bovier *et al.* [34] reported that, in a rat model of anxiety with electrical stimulation of the periaqueductal gray region, progabide, a GABA agonist, and diazepam increased the escape threshold, and a blockade of the GABA receptors by bicuculline reduced or abolished the action of progabide and diazepam. These results may be interpreted as an indication that GABAergic and/or serotonergic mechanisms are related to an anti-aversive action of benzodiazepines.

Brandao *et al.* [40] suggested that benzodiazepine, similar to chlordiazepoxide, acts directly on the dorsal periaqueductal gray area by enhancing the inhibitory influence of endogenous GABA. In addition, Sandner *et al.* [41] showed that neuronal activity in the DCG was related to medial hypothalamic stimulation-induced effects and escape responses. The medial part of the hypothalamus connects to and interacts with the lateral part [42]. This finding may be explained by the facilitation of the lateral hypothalamic self-stimulation rewarding system and/or to the reduction of periventricular aversive activity in the brain.

Accordingly, the benzodiazepine effect on conflict behavior may also be related to the facilitation of the dopaminergic rewarding system in the lateral hypothalamus and/or to reductions in the serotonergic aversion system in the periventricular structure influenced by the facilitation by drugs of presynaptic inhibition of the GABAergic mechanism.

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