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Behavioral Suppression Using Intracranial Reward and Punishment: Effects of Benzodiazepines

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MORIYAMA, M., Y. ICHIMARU AND Y. GOMITA. *Behavioral suppression using intracranial reward and punishment: Effects of benzodiazepines.* PHARMACOL BIOCHEM BEHAV **21**(5) 773–778, 1984.—Rats were chronically implanted with electrodes aimed at the lateral hypothalamus (LH) and the dorsal central gray (DCG) and trained to press a lever that delivered rewarding stimulation of the LH and punishing stimulation of the DCG. In this situation, both diazepam (5–20 mg/kg, PO) and bromazepam (2–10 mg/kg, PO) caused a marked dose-dependent increase of the lever pressing response in the punished period. In addition, the facilitation of lever pressing in unpunished period was also seen in diazepam (5 and 10 mg/kg). These results show that behavioral suppression on lever pressing maintained self-stimulation reward is inducible following DCG stimulation, and that benzodiazepines exhibit an anti-behavioral suppression effect in this situation.

"Conflict" situation Hypothalamic self-stimulation Dorsal central gray stimulation Benzodiazepines

IN order to measure the antianxiety activity of drugs in animals, various behavioral suppression tests involving "conflict," "punishment" or "passive avoidance" have been developed [2, 3, 4, 12]. Geller and Seifter [4] established a "conflict" situation by punishing with foot shock the lever pressing behavior of hungry rats pressing for food. Benzodiazepines and other minor tranquilizers were found to greatly reduce the suppressive effect of the foot shock [5]. Aversion can be induced not only by peripheral stimulation like foot shock but also by intracranial stimulation from electrodes in the periventricular system (dorsal central gray, ventromedial hypothalamus, raphe nuclei and so on [8, 13, 16, 21, 25]). Animals can be trained to terminate stimulation at these sites by pressing a lever. Graeff and Rawlins [9] recently reported that behavioral suppression could be induced by combining the punishment of dorsal periaqueductal gray stimulation with food reward, and that chlordiazepoxide antagonized the suppression.

All of these methods, however, use long-term hunger conditions, which create certain problems in assessing the activity of antianxiety drugs. The maintenance of good health in the animals is somewhat difficult and gastrointestinal drug absorption may be altered in such a deprived state. In a different approach, Gomita and Ueki [7] created a "conflict" situation by combining foot shock punishment and intracranial self-stimulation of the lateral hypothalamus (LH) and showed that antianxiety drugs antagonized the suppression in a dose-dependent fashion.

The purpose of the present study was to determine whether behavioral suppression could be produced by combining the punishment of midbrain dorsal central gray (DCG) stimulation with lever pressing for rewarding intracranial stimulation of the LH, and to investigate the effects of antianxiety drugs in this situation.

METHOD

Animals

Twelve male rats of the Wistar strain weighing 250–300 g at the beginning of the experiment were used as subjects. They were housed two per cage, in 26×36×25 cm plastic walled cages, and were given food and water ad lib throughout the experiment. The animals were maintained on a 12 hr light-dark cycle (lights on from 08:00 to 20:00) and at a room temperature of 22–24°C with a relative humidity of 60%.

Surgery and Histology

All animals were anesthetized with sodium pentobarbital 45 mg/kg, IP, and placed on stereotaxic instrument (Takahashi). At first, bipolar stainless steel electrodes (250 μm in diameter, insulated except at the tip) were chronically implanted into the lateral posterior hypothalamus (A: 5.8, L: 1.8, H: −2.5 mm) according to the stereotaxic coordinates of König and Klippel's brain atlas [14]. Rats that learned to press a lever for self-stimulation reward on a continuous reinforcement (CRF) schedule received a second electrode, implanted in the DCG (A: 0.6, L: 0.6, H: 0.4 mm), again under sodium pentobarbital anesthesia. In case of the DCG electrode implantation, the electrodes were bilaterally inserted into the target sites at a 15° angle in order to avoid piercing

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the sagittal venous sinus. At the end of the experiment, each animal was given an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline and 10% formalin. The brain was removed and immersed in formalin-saline solution for at least one week. The 40 μm slices were made after each brain was frozen in a cryostat (Chiyoda) following staining with cresylviolet. The localization of the implanted electrode tip in the lateral hypothalamus and DCG was then verified by inspection of the stained sections.

Apparatus

The experiments were carried out in a Skinner box, which was constructed of transparent Plexiglas with inside dimensions of 30 cm wide, 27 cm high and 25 cm deep. The floor was a stainless steel grid made of bars 5 mm in diameter and spaced 1.0 cm apart to allow urine and feces to fall through to the tray underneath. A lever was placed 4.5 cm above the grid floor and protruded 2.5 cm into the box. A small red lamp was provided near the lever as a cue light. A swivel was mounted in the ceiling of the chamber to hold the electrode lead so as to allow the animal free movement. Stimulations of the lateral hypothalamus and DCG were derived from a sine-wave stimulator (model-2305, Tohokosan) and a square-wave stimulator (MES-3R, Nihon Koden), respectively. The stimulators were controlled by programming circuitry and responses were recorded on a cumulative recorder (Gerbrand).

Procedure

After 7 or more days of recovery from implantation surgery, each animal was placed in the Skinner box and the stimulating cable was connected to the electrode plug mounted in the animal's head. All animals were first trained to press the lever for rewarding stimulation of the LH. The stimulation consisted of 60 Hz sinusoidal current lasting for 0.2 sec. Current was individually adjusted for each rat. The training was performed on a CRF schedule. The current was gradually increased, until the animal began to respond at a heightened activity level. A number of training sessions (15 min) were given to each animal daily. The current intensity (ranging from 20 to 100 μA) was adjusted to the level that supported maximum response rate of self-stimulation without gross motor disturbances or convulsion.

After the lever pressing for brain stimulation reward reached the high-rate criterion (1500 responses per 15 min) on three successive days, the other electrode was implanted in the DCG under sodium pentobarbital anesthesia, as described above. After recovery from the surgery, the DCG was stimulated. DCG stimulation consisted of negative square wave current ranging 20 to 120 μA at 100 Hz (0.1 msec pulse duration) lasting for 0.2 sec, and was given to each rat until it pressed a lever to stop the stimulation. DCG-stimulated animals showed aversive behavior such as defecation, rapid running and jumping, and learned to press the lever to escape this DCG-stimulation within one or two days. Only animals showing escape responses were used in the "conflict" experiment.

Thereafter, aversive stimulation of the DCG was combined with the self-stimulation. The self-stimulation reward —DCG stimulation aversion ("conflict") procedure was the same as the method of Gomita and Ueki [7] except that they used foot shock punishment. The test session consisted of a 15-min period, in which a 12-min unpunished period was followed by a 3-min punished period. The punished period was accompanied by a 1850 KHz tone and lighting of the cue light near the lever, where every response was rewarded with brain stimulation and simultaneously punished with a brief DCG electric stimulation. The intensity of the DCG stimulation was gradually increased in each animal until the response rate in the punished period was suppressed to less than 10, while the unpunished responding under the CRF schedule remained at a relatively high level. After the responses in each period were stable for three successive days, the animals were administered drugs. The drug test was performed at 1, 2, 4 and 24 hr postinjection. The animals were rested for at least 10 days between drug administrations.

Statistical Analysis

The experimental results were evaluated statistically by means of the Mann-Whitney U test [23].

Drugs

Drugs used in present experiment were diazepam (Kodama) and bromazepam (Kodama). Both of the drugs were suspended in 0.5% carboxymethylcellulose (CMC) suspension and administered orally. Control animals were given 0.5% CMC solution 0.1 ml per 100 g body weight.

RESULTS

Histology

Figure 1 illustrates the electrode tip placements in the posterior LH and the DCG. All electrode tips for self-stimulation were located in or on the border of the medial forebrain bundle at the posterior hypothalamus, and those for aversive brain stimulation in or adjacent to the DCG.

Effect of DCG Stimulation on Self-Stimulation

A stimulation reward delivered to the posterior LH with currents ranging 20 to 100 μA induced a rate of lever pressing in excess of 1500 responses per 15 min in all animals used in this study. These animals sometimes showed exploratory behavior as well as oral behavior. The DCG stimulation at low intensity (40 to 80 μA) frequently caused defecation, urination and rapid running behavior, and at high intensity ranging from 80 to 120 μA caused jumping to the ceiling of the box but without convulsion. Rats showing the above behavior learned to press a lever to stop the aversive brain stimulation with one or two days of training. After a stable high response rate on self-stimulation was obtained under the CRF schedule through three successive days, the DCG stimulation was combined with the self-stimulation, and the stimulus intensity for DCG stimulation in the punished period was gradually increased. In this period, an approach-avoidance behavior was induced and the lever pressing was reduced in an intensity dependent manner, i.e., a "conflict" situation was established, as shown in Fig. 2.

Effects of Drugs

The effects of benzodiazepines, diazepam and bromazepam, were investigated in the 7 rats showing the most stable performance in the "conflict" situation. The effects of diazepam (20 mg/kg) and bromazepam (10 mg/kg) in each representative rat are shown in the cumulative records of Figs. 3 and 4, respectively. In Fig. 3, diazepam at a dose of 20 mg/kg, caused a marked increase in lever pressing during the punished period without affecting the unpunished rate.

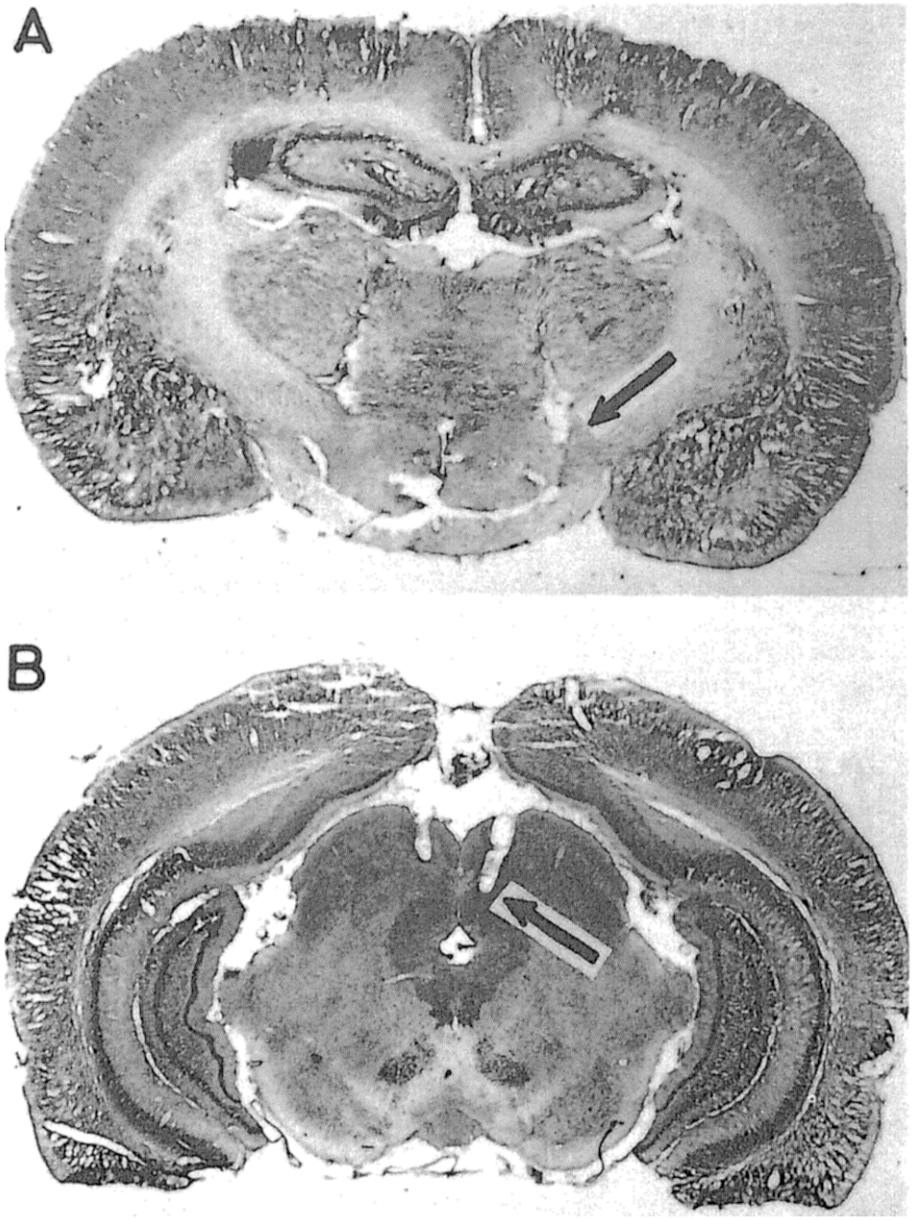

FIG. 1. Nissl-stained coronal sections showing the tips of the electrode in lateral hypothalamus (A) and dorsal central gray (B) for stimulation.

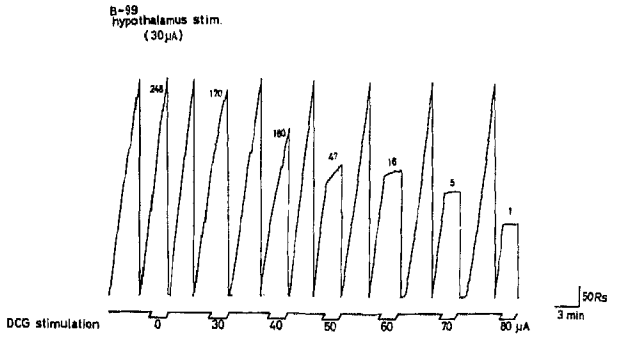

FIG. 2. Cumulative response records for a representative rat (B–99) showing the effect of contingent dorsal central gray (DCG) stimulation (30–80 μA) on lever pressing maintained by rewarding electrical stimulation of the lateral hypothalamus (30 μA). Ordinate: responses; Abscissa: time. The punished period (2 min) is indicated on the lower line. The numbers in the cumulative record indicate the responses for lever pressing during the punished period.

The effect appeared within 1 hr, reached its maximum at 1 hr and lasted until 1 to 2 hr after administration. Bromazepam at a dose of 10 mg/kg also caused a marked increase of the lever pressing response in punished period as shown in Fig. 4. This appeared within 1 hr and lasted until 1 to 4 hr after drug administration. Figure 5 shows the mean lever pressing responses in the punished and unpunished periods after administration of diazepam or bromazepam at various doses in each group of 4 to 6 rats. Diazepam at doses of 5–20 mg/kg caused a marked dose-dependent increase in lever pressing during the punished period and at doses of 5–10 mg/kg caused a slight increase during the unpunished period as well. Significant differences in responding during the punished period were found with diazepam at 1 hr after administration of 5 mg/kg ($U=1$, $p<0.01$) and 10 mg/kg ($U=0$, $p<0.01$), and at 1 ($U=0$, $p<0.01$) and 2 hr ($U=2$, $p<0.05$) after 20 mg/kg administration. During the unpunished period with diazepam, significant differences were found at 1 hr ($U=1.5$, $p<0.05$) after 5 mg/kg administration and at 1 ($U=5$, $p<0.05$) and 4 hr ($U=4$, $p<0.05$) after 10 mg/kg administration. In bromazepam (2–10 mg/kg) treated groups, a marked dose-dependent increase in lever pressing during the punished period was observed. Significant differences were found with bromazepam at 1 ($U=1$, $p<0.05$) and 2 hr ($U=0$, $p<0.01$) after 2 mg/kg administration, at 1 ($U=1$, $p<0.05$) and 2 hr ($U=2$, $p<0.05$) after 5 mg/kg administration, and at 1 ($U=0$, $p<0.01$), 2 ($U=0$, $p<0.01$) and 4 hr ($U=0$, $p<0.01$) after 10 mg/kg administration.

DISCUSSION

Our results show that suppression of lever pressing is seen when there is a "conflict" between contingent rewarding stimulation of the LH and simultaneous contingent aversive stimulation of the DCG. Benzodiazepines, diazepam and bromazepam, dramatically antagonize the suppression effect. This procedure for behavioral suppression was the same as that of Geller and Seifter [4], except that they used food reward and foot shock aversion. The results obtained with benzodiazepines in this experiment are almost the same as the effects of benzodiazepines on a "conflict" situation by combining the foot shock aversion with food [5] or self-stimulation reward [7].

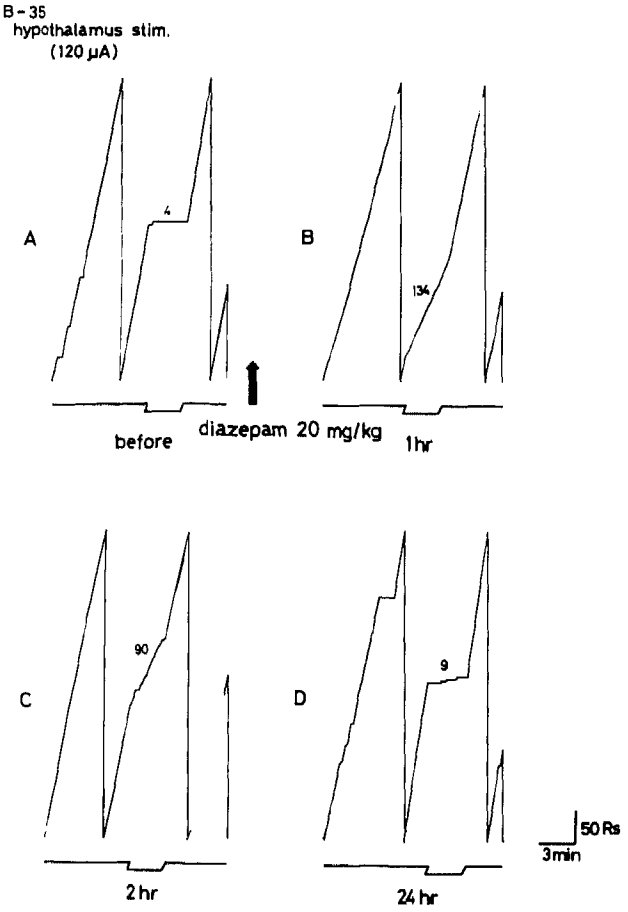

FIG. 3. Effect of diazepam on a "conflict" situation induced by combining lateral hypothalamic self-stimulation reward with dorsal central gray stimulation aversion. Cumulative recording of the lever pressing before (A), 1 hr (B), 2 hr (C) and 24 hr (D) after administration of diazepam 20 mg/kg PO. The punished period (3 min) is indicated on the lower line in each panel. The number of lever presses in the punished period is included in the figure.

Benzodiazepines increase the low rates of lever pressing maintained by DRL or VI procedures with food reward [19, 20, 22] or brain stimulation reward [6,11]. The effective doses of benzodiazepines in our situation using LH self-stimulation reward and DCG stimulation aversion were markedly lower than in situations involving DRL responding, or in "conflict" situations involving conventioned reinforcements such as food and milk [5]. And further, the drug effects in this situation were more sensitive in comparison with those in the situation using intracranial reward and foot shock [7]. Benzodiazepines are said to act not by increasing the appetitive motivation leading to behavior, but rather by releasing behavioral suppression, i.e., by disinhibitory action [15]. Recently reported results have shown that benzodiazepines facilitate GABAergic synaptic mechanisms, which secondarily cause a reduction of serotonergic activity [24]. To explain the enhanced effectiveness of benzodiazepines when lateral hypothalamic reward is used, we have suggested in a previous report that GABA-mediated neurons facilitated by benzodiazepines may increase the activity of dopaminergic neurons in the reward system [11].

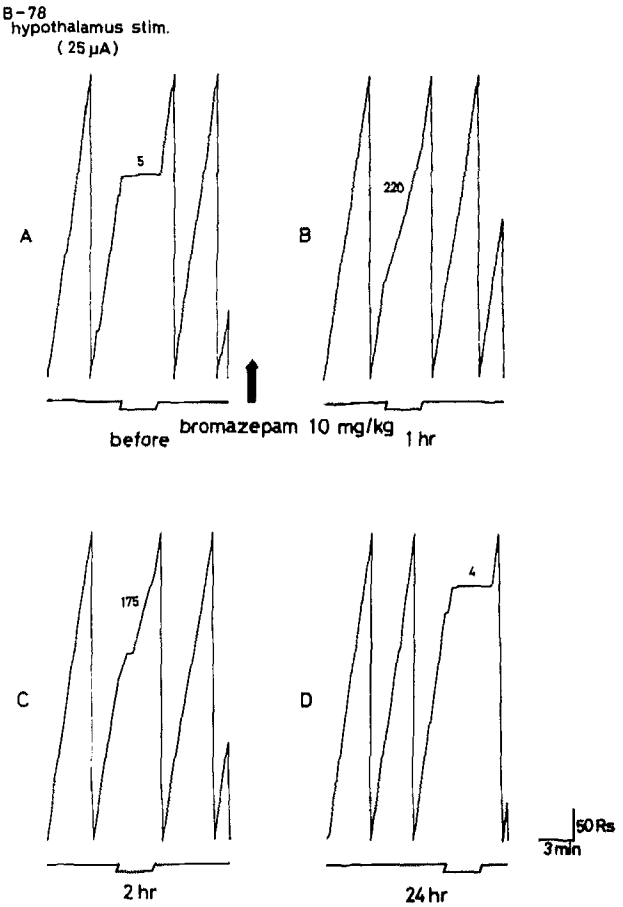

FIG. 4. Effect of bromazepam on a "conflict" situation obtained by combining lateral hypothalamic self-stimulation reward with dorsal central gray stimulation aversion. Cumulative recording of the lever pressing before (A), 1 hr (B), 2 hr (C) and 24 hr (D) after administration of bromazepam 10 mg/kg PO. The punished period (3 min) is indicated in the lower line in each panel. The number of lever presses in the punished period is included in the figure.

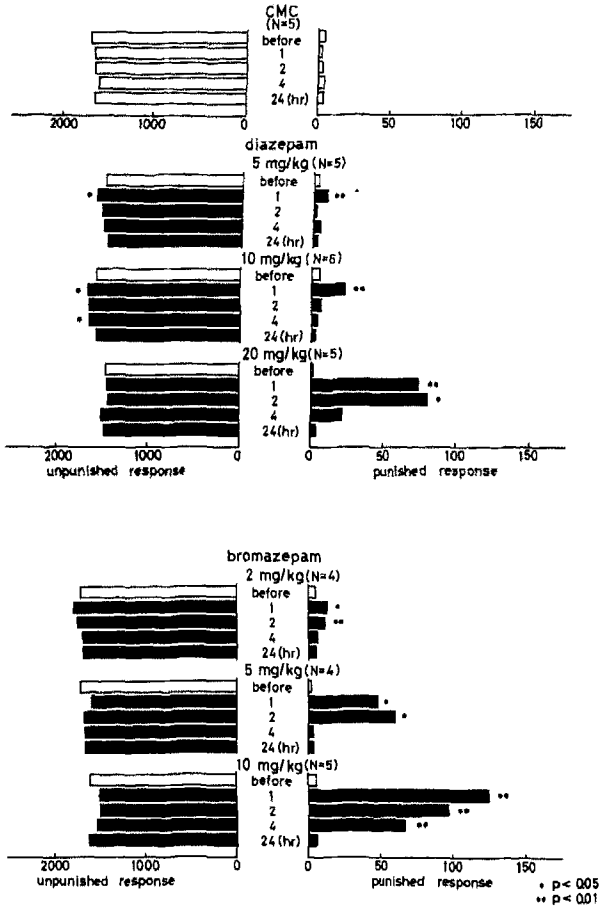

FIG. 5. Effects of diazepam and bromazepam on punished (3-min period) and unpunished responding (12-min period). Asterisks indicate significant differences from the value of the CMC administered group.

On the other hand, Brandao *et al.* [1] have suggested that chlordiazepoxide acts directly upon the dorsal periaqueductal gray by enhancing the inhibitory influence of endogenous GABA. The mesencephalic central gray and the medial hypothalamus are known as the periventricular system, which mediates aversive behavior induced by brain stimulation [18,21]. In addition, Sandner *et al.* [17] 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 [10]. Thus our results may be attributed to facilitation of the LH self-stimulation rewarding system and/or to the reduction of periventricular aversive activity in the brain. 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 reduction of the serotonergic aversion system in the periventricular structure influenced by the facilitation of presynaptic inhibition of GABAergic mechanism by drugs.

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