Chlordiazepoxide, Go-Nogo Successive Discrimination and Brain Biogenic Amines in Cats[1]

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VACHON, L., A. KITSIKIS AND A. G. ROBERGE. *Chlordiazepoxide, go-nogo successive discrimination and brain biogenic amines in cats.* PHARMACOL BIOCHEM BEHAV **20**(1) 9–22, 1984.—Chlordiazepoxide (CDP; 0.4 mg/kg/day, per os) was administered to cats during either the acquisition (CDP 21–22 days) of a go-nogo successive discrimination task (SD) or the performance (CDP 10 days) of the previously learned SD task. Endogenous levels of serotonin, 5-hydroxyindoleacetic acid, noradrenaline and dopamine were assayed in 12 brain areas, in trained as well as in untrained cats. This study has shown that (1) CDP strongly impaired the acquisition but not performance of the SD task, revealing a dissociation of the effects of CDP on these two stages of training; (2) the CDP administration, as well as the SD training, produced regional changes in brain levels of biogenic amines, suggesting the involvement of particular monoaminergic neurons in the behavioral effects of CDP and in operant behavior; and (3) in particular brain areas, interactions were observed between the effects of the SD training and those of the CDP administration on monoamines, indicating that the behavioral state may interfere with the neurochemical effects of CDP.

Benzodiazepines   Chlordiazepoxide   Acquisition   Performance   Go-nogo
Successive discrimination   Biogenic amines   Serotonin   Noradrenaline   Dopamine   Cats

SEVERAL investigators [13, 23, 29, 30, 42, 52, 72, 75] have reported that benzodiazepines (BZP) impair acquisition or performance of various discrimination tasks in animals. These effects have been tentatively related to a BZP-induced disinhibition of certain response patterns. However, Saghal and Iversen [50] have postulated that chlordiazepoxide (CDP) impairs discrimination performance by disrupting encoding processes. Soubrié *et al.* [56] have also proposed that BZP induce amnesia by interfering with registration mechanisms. Moreover, in man, low doses of diazepam have been reported to impair acquisition of new information without impairing retrieval processes [15, 25, 36].

Many studies have also shown that BZP reduce the utilization of serotonin (5-HT), noradrenaline (NA) and dopamine (DA) in the central nervous system (CNS) [60]. It has been suggested that these biochemical modifications are involved in the behavioral effects of BZP [14, 47, 54, 60, 69]. However, the exact psychopharmacological and neuroanatomical basis of the BZP-induced effects on brain monoamines still remain to be clarified. Moreover, in most of these biochemical studies, large doses of BZP were used

and the effects of these drugs on biogenic amines were observed in the whole brain or in a few brain areas.

The present study was undertaken in order to precise the biochemical mechanisms and the neuroanatomical structures involved in the effects of a clinically relevant dose of CDP (0.4 mg/kg) on operant behavior. In addition, the neurochemical effects of training were investigated, as previous reports have shown that training may produce changes in the metabolism of 5-HT, NA and DA in the CNS of normal animals [9, 11, 24, 35, 68], thus suggesting the involvement of biogenic amines in operant behavior. Two experiments were therefore designed to observe, in cats, (1) the effects of clinically relevant dose of CDP (0.4 mg/kg/day, per os) on either the acquisition or the performance of a visual go-nogo successive discrimination task (SD) with positive symmetrical reinforcement; (2) the effects of a long-term administration of CDP (21–22 days or 10 days) on brain endogenous levels of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), NA and DA in 12 brain areas; (3) the effects of the SD acquisition and performance on brain biogenic amines; and (4) the interactions between the neurochemical effects of the SD training and those of the CDP administration.

GENERAL METHOD

Animals

Forty-six adult mongrel cats of both sexes, initially weighing 2.8±0.1 kg, were used in this experiment. They were housed in individual cages with room temperature at 21°C and humidity at 55%. Background music was broadcasted between 7:00 and 19:00 hr and a 12 hr light-dark cycle (light on 7:00–19:00 hr) was enforced. Cats were fed with Laboratory Cat Chow, taking into account the amount of meat received during the training session in order to maintain a constant body weight (100±3%) throughout the experiment, and had free access to water. All animals were regularly examined by a veterinarian and adapted to the environmental conditions for at least two weeks prior to the experiments, which were conducted during the winter.

Both Experiments 1 and 2 included the following 4 groups of cats, equally distributed in respect to sex and weight: (1) untrained, manipulated controls; (2) untrained, manipulated cats treated with CDP; (3) controls trained on SD; and (4) cats treated with CDP as group 2 and trained on SD as group 3.

Behavioral Procedure

The experimental set-up consisted of a wooden box equipped with a one-way screen, in front of which was placed a tray with a centrally situated food-well covered by a wooden block. A black cover represented the positive stimulus (go) and a white cover the negative stimulus (nogo).

All experimental animals first underwent a 2-day shaping period during which they became habituated to the training apparatus and learned to uncover the food-well for meat reinforcement. During shaping, a natural colored wooden block was used and animals did not perform more than 4 complete trials before the onset of training, which was carried out between 8:00 and 12:00 hr. Cats were trained in a different order each day to reduce interference of the circadian cycle in the training schedule. Fifty trials (25 go and 25 nogo) were given in each session in a randomized sequence defined to avoid the same situation on more than 3 consecutive trials. On each trial, the unidirectional screen was lifted, exposing either a black or white wooden block covering the food-well. On go trials, animals were required to remove the clock within 5 sec in order to retrieve a small piece of meat from the food-well. On nogo trials, cats had to refrain from displacing the block for 7 sec before being reinforced with a small piece of meat by the experimenter. Intertrial intervals lasted 10 sec. Response latencies, defined as the period that elapsed between the time the screen was lifted and the moment the cat's paw touched the wooden block covering the food-well, were recorded by the experimenter.

Immediately after the daily training session of a trained animal (groups 3 and 4), an untrained cat of either group 1 or group 2 was placed in the experimental box. The subject then received the same quantity of meat as the preceding trained animal and remained in the apparatus until all the meat had been eaten. The amount of meat consumed varied between 25 and 48 g, depending on performance of trained cats. This procedure was carried out in order to keep animals in comparable experimental and dietary conditions.

Sacrifices were performed between 8:30 and 10:30 hr, the animals being killed within 5 min after being taken out of the apparatus. In order to minimize the effect of circadian cycle fluctuations, the sacrifices of cats belonging to the 4 groups were alternated.
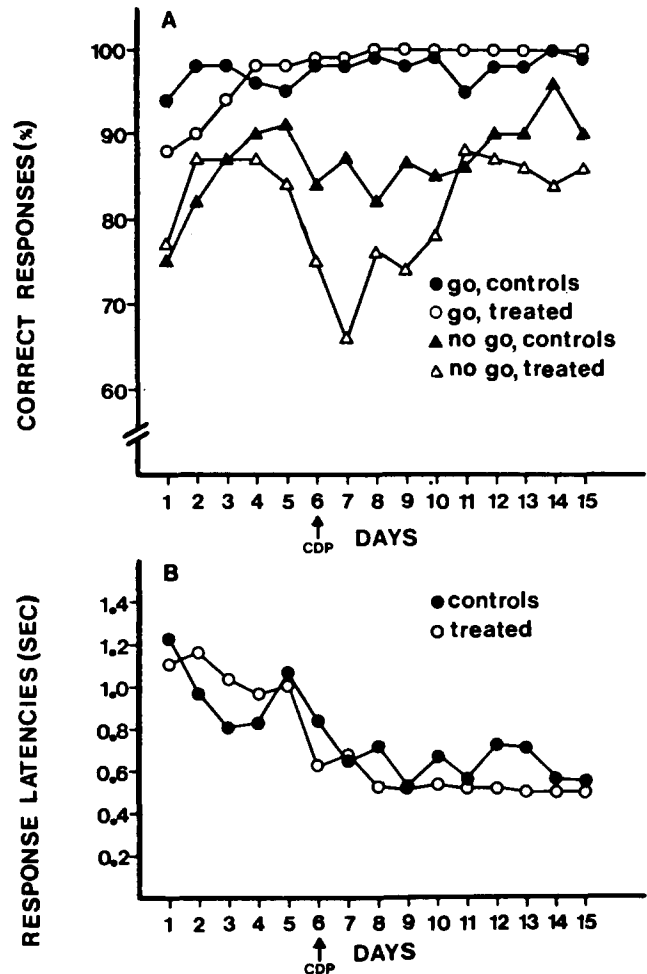

FIG. 1. Effects of chlordiazepoxide (0.4 mg/kg/day) on performance (% of correct responses) on go and nogo trials (A), and on response latencies (sec) on correct go trials (B) of cats trained on a go-nogo successive discrimination task for 15 days.

CDP Administration

CDP (0.4 mg/kg, per os) was administered in gelatine capsules daily, 7 days a week, between 14:00 and 14:30 hr. Controls (group 1 and 3) received empty capsules at the same time. On the day before sacrifice, subjects were given their last CDP administration at 10 min intervals, beginning at 14:00 hr, and were killed 18 hr later.

Biochemical Assays

Cats were decapitated without anesthesia, using a guillotine specially designed for this purpose. Brains were immediately set apart and the following structures were dissected out on ice and kept frozen at −80°C until biochemical assays were performed: the frontal cortex, neostriatum, septum, thalamus, hypothalamus, piriform lobe (amygdala), hippocampus, the dorsal and ventral mesencephalic areas (deprived of raphe nuclei), pons (deprived of raphe nuclei), medulla (deprived of raphe nuclei), and the respective raphe nuclei of the mesencephalon, pons and medulla. 5-HT, 5-HIAA, NA and DA were isolated using Sephadex G-10 as described by Earley and Leonard [10], with slight modifica-

tions. 5-HT and 5-HIAA were determined according to Curzon and Green [5]. NA was oxidized using the technique of Maickel *et al.* [39], with minor modifications, whereas DA was determined according to a slightly modified method of Welch and Welch [73]. Fluorescence was read using an Aminco-Bowman spectrophotofluorimeter (American Instruments Co. Inc., Silver Springs, MD). Recoveries of 5-HT, 5-HIAA, NA and DA were $68\pm2\%$, $76\pm2\%$, $88\pm2\%$ and $86\pm2\%$, respectively. All estimates were corrected for losses.

Statistical Analysis

Behavioral data were analysed using a split-plot factorial design as described by Kirk [34]. Biochemical data were analyzed using a factorial analysis for unequal groups [22], in order to assess the neurochemical effects of the SD training (A), the neurochemical effects of the CDP administration (B), and the A×B interactions. When a significant SD training × CDP administration interaction was observed, simple effects were assessed using the test of Bayes for multiple comparisons with unequal groups, as described by Smith [55]. Standard error of the mean and the t-test were calculated according to Lison [38].

Chemicals

5-HT (5-hydroxytryptamine, creatinine sulfate complex), 5-HIAA (5-hydroxyindole-3-acetic acid, dicyclohexylammonium salt), NA (DL-arterenol HCl), DA (3-hydroxytyramine HCl) and Sephadex G-10 were from Sigma Chemical Co., St. Louis, MO. Columns (glass-barrell ECONO-columns, i.d. 7 cm×40 mm) were obtained from BIO-Rad Laboratories Ltd, Mississauga, Ontario, Canada. Gelatine capsules (No. 0) were purchased from Parke, Davis and Company Ltd, Brockville, Ontario, Canada. Chlordiazepoxide HCl was kindly provided by Hoffman-La Roche Ltd, Vaudreuil, Québec, Canada.

EXPERIMENT 1: EFFECTS OF CHLORDIAZEPOXIDE ON GO-NOGO ACQUISITION AND BRAIN BIOGENIC AMINES

METHOD

Animals were assigned to 4 groups of 6 cats. Group 1 consisted of untrained, manipulated controls, while animals in group 2 were untrained, manipulated cats treated with CDP (0.4 mg/kg/day, per os) for 21–22 days. Cats in group 3 were trained on SD for 15–16 days. Group 4 included animals trained on SD as group 3 and receiving CDP as group 2.

The CDP administration began 2 days before the shaping period and was given throughout the SD acquisition, the animals being trained 18 hr after the CDP administration. CDP was given 7 days a week, and the training was carried out 6 days a week. On the 15th and 16th training days, 12 cats—3 subjects from each of the 4 groups—were killed. As animals were sacrificed on 2 consecutive days, a total of 21 CDP administrations were given to 12 cats and 22 to the other 12 cats.

RESULTS

Behavioral Data

In Fig. 1A, the effects of the CDP administration on performance (percentage of correct responses) of cats are shown in go and nogo trials for the 15 days of training. On go trials, performance of controls ($97.6\pm1.1\%$) and CDP treated cats ($99.8\pm0.2\%$) was not different. There was no effect of CDP, $F(1,10)=4.52$, but a significant effect of days, $F(14,140)=1.91$, $p<0.05$, was observed. However, there was no CDP administration × days interaction, $F(14,140)=0.88$, NS. On nogo trials, performance (controls $54.9\pm3.1\%$, CDP treated cats $=11.6\pm9.4\%$) was significantly affected by CDP. A CDP effect, $F(1,10)=22.82$, $p<0.01$, a days effect, $F(14,140)=26.02$, $p<0.01$, and a CDP administration × days interaction, $F(14,140)=16.76$, $p<0.01$, were observed. Performance of CDP treated cats was significantly lower than in controls on the 6th ($p<0.05$), and on the 7th to the 15th ($p<0.01$) training days (Fig. 1A). In addition, the results show that performance of controls varied significantly, $F(14,140)=41.86$, $p<0.01$, during training, whereas performance of CDP treated cats did not change significantly, $F(14,140)=0.91$, NS, through the 15 days of training.

Figure 1B illustrates the response latencies of controls (0.85 ± 0.15 sec) and CDP treated cats (0.57 ± 0.19 sec) on correct go trials. In spite of the fact that a strong difference in response latencies was observed between the 2 groups, such a difference being due to the response latencies of 2 controls, no effect of CDP, $F(1,10)=3.88$, NS, was observed. However, there was a significant effect of days, $F(14,140)=4.77$, $p<0.01$, but no CDP administration × days interaction, $F(14,140)=0.83$, NS.

Biochemical Data

Tables 1–5 (left part) show the endogenous levels of 5-HT and 5-HIAA, the 5-HT:5-HIAA ratio, and the concentrations of NA and DA, observed in 12 brain areas in the 4 experimental groups. These tables present (1) the neurochemical effects of the SD training (A); (2) the neurochemical effects of the CDP administration (B); and (3) the interactions between the effects of the SD training and those of the CDP administration (I).

The SD acquisition induced a significant decrease in the 5-HT content in the frontal cortex ($p<0.05$), whereas significant increases were observed in the thalamus ($p<0.05$), hypothalamus ($p<0.05$), ventral mesencaphalon ($p<0.05$) and mesencephalon raphe nuclei ($p<0.01$) (Table 1). The 5-HIAA level was enhanced significantly by the SD acquisition in the frontal cortex ($p<0.01$), thalamus ($p<0.05$), piriform lobe ($p<0.05$), ventral mesencephalon ($p<0.01$), pons-medulla ($p<0.05$), mesencephalon raphe nuclei ($p<0.05$) and pons-medulla raphe nuclei ($p<0.05$) (Table 2). As shown in Table 3, the SD acquisition led to a decrease of the 5-HT:5-HIAA ratio in the frontal cortex ($p<0.01$), whereas this ratio was enhanced significantly by training in the hypothalamus ($p<0.05$). The SD acquisition induced an increase in the NA concentration in the piriform lobe ($p<0.05$) and mesencephalon raphe nuclei ($p<0.05$) (Table 4). In addition, the DA content was raised significantly by the SD acquisition in the neostriatum ($p<0.05$) and dorsal mesencephalon ($p<0.01$) (Table 5).

As shown in Table 1, the 21–22 days CDP administration induced an increase in the 5-HT level in the septum ($p<0.05$), hypothalamus ($p<0.05$) and ventral mesencephalon ($p<0.01$). The 5-HIAA concentration was lowered significantly by the CDP administration in the thalamus ($p<0.01$), hippocampus ($p<0.01$), piriform lobe ($p<0.01$) and pons-medulla raphe nuclei ($p<0.05$) (Table 2). The CDP administration led to a rise of the 5-HT:5-HIAA ratio in the septum ($p<0.01$), thalamus ($p<0.01$), hypothalamus ($p<0.01$) and

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE (CDP; 0.4 mg/kg/DAY, PER OS) ON THE SEROTONIN CONTENT IN BRAIN OF UNTRAINED
CATS AND CATS TRAINED ON A GO-NOGO SUCCESSIVE DISCRIMINATION TASK

| | Experiment 1 Go-Nogo Acquisition—CDP 21–22 Days | | | | | | |
| | Untrained | | Trained | | Factorial‡ analysis | | |
Structures†	Controls (6)	CDP (6)	Controls (6)	CDP (6)	A	B	I
Frontal cortex	0.22 ± 0.02	0.21 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	*	NS	NS
Neostriatum	0.56 ± 0.06	0.40 ± 0.03	0.51 ± 0.14	0.49 ± 0.05	NS	NS	NS
Septum	0.86 ± 0.19	1.24 ± 0.16	1.00 ± 0.10	1.20 ± 0.09	NS	*	NS
Thalamus	0.43 ± 0.07	0.53 ± 0.06	0.66 ± 0.07	0.61 ± 0.04	*	NS	NS
Hypothalamus	0.65 ± 0.08	0.78 ± 0.06	0.78 ± 0.06	0.95 ± 0.08	*	*	NS
Hippocampus	0.42 ± 0.03	0.42 ± 0.04	0.41 ± 0.04	0.48 ± 0.04	NS	NS	NS
Piriform lobe (amygdala)	0.81 ± 0.07	0.73 ± 0.09	0.86 ± 0.07	0.77 ± 0.06	NS	NS	NS
Dorsal mesencephalon§	0.86 ± 0.05	0.98 ± 0.12	1.02 ± 0.12	1.13 ± 0.08	NS	NS	NS
Ventral mesencephalon§	0.76 ± 0.06	0.92 ± 0.08	0.89 ± 0.05	1.12 ± 0.06	*	**	NS
Pons-medulla§	0.68 ± 0.08	0.71 ± 0.08	0.76 ± 0.05	0.69 ± 0.06	NS	NS	NS
Raphe nuclei (mesencephalon)	0.99 ± 0.15	1.34 ± 0.22	1.61 ± 0.20	1.69 ± 0.10	**	NS	NS
Raphe nuclei (pons-medulla)	0.53 ± 0.07	0.59 ± 0.04	0.77 ± 0.05¶	0.62 ± 0.06	—	—	*

†Results are expressed in μg/g (fresh tissue) (Mean ± SEM). Number of animals is in brackets.
‡A: effects of go-nogo training; B: effects of CDP administration; I: A×B interactions; $*p<0.05$; $**p<0.01$.
§Without raphe nuclei.
¶$p<0.05$; comparison with untrained controls.

TABLE 2

EFFECTS OF CHLORDIAZEPOXIDE (CDP; 0.4 mg/kg/DAY, PER OS) ON THE 5-HYDROXYINDOLEACETIC ACID CONTENT IN BRAIN
OF UNTRAINED CATS AND CATS TRAINED ON A GO-NOGO SUCCESSIVE DISCRIMINATION TASK

| | Experiment 1 Go-Nogo Acquisition—CDP 21–22 Days | | | | | | |
| | Untrained | | Trained | | Factorial‡ analysis | | |
Structures†	Controls (6)	CDP (6)	Controls (6)	CDP (6)	A	B	I
Frontal cortex	0.33 ± 0.02	0.35 ± 0.02	0.41 ± 0.02	0.39 ± 0.02	**	NS	NS
Neostriatum	1.08 ± 0.09	0.96 ± 0.13	1.03 ± 0.18	0.96 ± 0.04	NS	NS	NS
Septum	1.12 ± 0.18	0.98 ± 0.05	1.21 ± 0.17	1.09 ± 0.08	NS	NS	NS
Thalamus	0.84 ± 0.08	0.69 ± 0.05	1.05 ± 0.10	0.76 ± 0.05	*	**	NS
Hypothalamus	1.87 ± 0.23	1.75 ± 0.16	2.03 ± 0.12	1.70 ± 0.11	NS	NS	NS
Hippocampus	0.76 ± 0.04	0.62 ± 0.03	0.70 ± 0.06	0.60 ± 0.04	NS	**	NS
Piriform lobe (amygdala)	0.70 ± 0.04	0.60 ± 0.04	0.77 ± 0.05	0.68 ± 0.01	*	**	NS
Dorsal mesencephalon§	1.46 ± 0.14	1.04 ± 0.08	1.36 ± 0.15	1.55 ± 0.15	—	—	*
Ventral mesencephalon§	1.03 ± 0.08	1.08 ± 0.11	1.33 ± 0.12	1.45 ± 0.11	**	NS	NS
Pons-medulla§	0.58 ± 0.04	0.59 ± 0.04	0.71 ± 0.07	0.68 ± 0.07	*	NS	NS
Raphe nuclei (mesencephalon)	1.33 ± 0.17	1.21 ± 0.16	1.55 ± 0.12	1.74 ± 0.18	*	NS	NS
Raphe nuclei (pons-medulla)	1.11 ± 0.17	0.89 ± 0.07	1.34 ± 0.15	1.11 ± 0.04	*	*	NS

†Results are expressed in μg/g (fresh tissue) (Mean ± SEM). Number of animals is in brackets.
‡A: effects of go-nogo training; B: effects of CDP administration; I: A×B interactions; $*p<0.05$; $**p<0.01$.
§Without raphe nuclei.

TABLE 1

(Continued)

Experiment 2
Go-Nogo Performance—CDP 10 Days

| Untrained | | Trained | | Factorial* analysis | | |
Controls (6)	CDP (6)	Controls (5)	CDP (5)	A	B	I
0.30 ± 0.04	0.31 ± 0.02	0.32 ± 0.03	0.29 ± 0.03	NS	NS	NS
0.76 ± 0.07	0.83 ± 0.08	0.74 ± 0.11	0.62 ± 0.09	NS	NS	NS
1.23 ± 0.09	1.51 ± 0.16	1.22 ± 0.12	0.26 ± 0.14	NS	NS	NS
0.46 ± 0.05	0.44 ± 0.04	0.47 ± 0.05	0.43 ± 0.05	NS	NS	NS
1.07 ± 0.07	0.98 ± 0.07	0.89 ± 0.09	1.10 ± 0.05	—	—	*
0.52 ± 0.04	0.64 ± 0.02	0.64 ± 0.10	0.73 ± 0.08	NS	NS	NS
0.93 ± 0.10	1.01 ± 0.16	0.91 ± 0.09	1.03 ± 0.08	NS	NS	NS
1.21 ± 0.14	1.21 ± 0.14	1.14 ± 0.09	1.25 ± 0.12	NS	NS	NS
1.02 ± 0.10	1.04 ± 0.11	1.18 ± 0.08	0.98 ± 0.09	NS	NS	NS
0.67 ± 0.06	0.78 ± 0.05	0.68 ± 0.03	0.81 ± 0.04	NS	*	NS
1.35 ± 0.09	1.18 ± 0.12	1.50 ± 0.08	1.41 ± 0.12	NS	NS	NS
0.69 ± 0.09	0.82 ± 0.09	0.85 ± 0.08	0.78 ± 0.08	NS	NS	NS

TABLE 2

(Continued)

Experiment 2
Go-Nogo Performance—CDP 10 Days

| Untrained | | Trained | | Factorial* analysis | | |
Controls (6)	CDP (6)	Controls (5)	CDP (5)	A	B	I
0.33 ± 0.03	0.38 ± 0.02	0.31 ± 0.04	0.31 ± 0.01	NS	NS	NS
1.32 ± 0.09	1.28 ± 0.07	1.24 ± 0.09	1.04 ± 0.06	*	NS	NS
1.91 ± 0.10	1.99 ± 0.20	1.78 ± 0.31	1.75 ± 0.16	NS	NS	NS
1.06 ± 0.06	0.86 ± 0.06	0.91 ± 0.10	0.82 ± 0.05	NS	*	NS
1.83 ± 0.11	1.67 ± 0.05	1.57 ± 0.16	1.72 ± 0.05	NS	NS	NS
0.80 ± 0.07	0.81 ± 0.05	0.72 ± 0.04	0.72 ± 0.04	NS	NS	NS
0.96 ± 0.05	0.95 ± 0.07	0.84 ± 0.04	0.89 ± 0.04	NS	NS	NS
1.14 ± 0.14	1.25 ± 0.12	0.99 ± 0.04	1.12 ± 0.11	NS	NS	NS
1.41 ± 0.09	1.41 ± 0.16	1.28 ± 0.12	1.19 ± 0.10	NS	NS	NS
0.74 ± 0.04	0.77 ± 0.07	0.60 ± 0.03	0.68 ± 0.04	*	NS	NS
2.09 ± 0.20	1.87 ± 0.21	2.29 ± 0.29	1.65 ± 0.09	NS	*	NS
1.14 ± 0.11	1.22 ± 0.15	1.17 ± 0.08	1.06 ± 0.10	NS	NS	NS

TABLE 3

EFFECTS OF CHLORDIAZEPOXIDE (CDP; 0.4 mg/kg/DAY, PER OS) ON THE SEROTONIN: 5-HYDROXYINDOLEACETIC ACID RATIO
IN BRAIN OF UNTRAINED CATS AND CATS TRAINED ON A GO-NOGO SUCCESSIVE DISCRIMINATION TASK

	Experiment 1						
	Go-Nogo Acquisition—CDP 21-22 Days						
	Untrained		Trained		Factorial‡ analysis		
Structures†	Controls (6)	CDP (6)	Controls (6)	CDP (6)	A	B	I
Frontal cortex	0.66 ± 0.03	0.62 ± 0.06	0.46 ± 0.02	0.49 ± 0.05	**	NS	NS
Neostriatum	0.51 ± 0.03	0.44 ± 0.06	9.50 ± 0.12	0.52 ± 0.05	NS	NS	NS
Septum	0.77 ± 0.11	1.26 ± 0.15	0.85 ± 0.08	1.14 ± 0.15	NS	**	NS
Thalamus	0.51 ± 0.07	0.76 ± 0.07	0.66 ± 0.10	0.81 ± 0.06	NS	NS	NS
Hypothalamus	0.35 ± 0.02	0.45 ± 0.03	0.39 ± 0.04	0.56 ± 0.04	*	**	NS
Hippocampus	0.56 ± 0.06	0.66 ± 0.04	0.60 ± 0.07	0.82 ± 0.07	NS	**	NS
Piriform lobe (amygdala)	1.15 ± 0.09	1.21 ± 0.09	1.13 ± 0.06	1.15 ± 0.09	NS	NS	NS
Dorsal mesencephalon§	0.61 ± 0.06	0.95 ± 0.11¶	0.76 ± 0.06	0.74 ± 0.05	—	—	*
Ventral mesencephalon§	0.75 ± 0.06	0.88 ± 0.10	0.69 ± 0.09	0.79 ± 0.07	NS	NS	NS
Pons-medulla§	1.18 ± 0.12	1.19 ± 0.10	1.10 ± 0.11	1.03 ± 0.06	NS	NS	NS
Raphe nuclei (mesencephalon)	0.76 ± 0.10	1.10 ± 0.06	1.04 ± 0.12	1.00 ± 0.09	NS	NS	NS
Raphe nuclei (pons-medulla)	0.50 ± 0.06	0.68 ± 0.05	0.59 ± 0.03	0.55 ± 0.06	—	—	*

†Results are expressed in μg/g (fresh tissue) (Mean ± SEM). Number of animals is in brackets.
‡A: effects of go-nogo training; B: effects of CDP administration; I: A×B interactions; $*p<0.05$; $**p<0.01$.
§Without raphe nuclei.
¶$p<0.05$; comparison with untrained controls.

TABLE 4

EFFECTS OF CHLORDIAZEPOXIDE (CDP; 0.4 mg/kg/DAY, PER OS) ON THE NORADRENALINE CONTENT IN BRAIN OF
UNTRAINED CATS AND CATS TRAINED ON A GO-NOGO SUCCESSIVE DISCRIMINATION TASK

	Experiment 1						
	Go-Nogo Acquisition—CDP 21-22 Days						
	Untrained		Trained		Factorial‡ analysis		
Structures†	Controls (6)	CDP (6)	Controls (6)	CDP (6)	A	B	I
Frontal cortex	0.24 ± 0.02	0.30 ± 0.04	0.26 ± 0.01	0.30 ± 0.02	NS	NS	NS
Neostriatum	0.26 ± 0.04	0.24 ± 0.05	0.22 ± 0.04	0.16 ± 0.02	NS	NS	NS
Septum	0.67 ± 0.12	0.87 ± 0.15	0.80 ± 0.09	0.92 ± 0.08	NS	NS	NS
Thalamus	0.31 ± 0.03	0.44 ± 0.05	0.49 ± 0.04	0.45 ± 0.03	—	—	*
Hypothalamus	1.85 ± 0.27	2.73 ± 0.31	2.29 ± 0.15	2.43 ± 0.16	NS	*	NS
Hippocampus	0.17 ± 0.02	0.27 ± 0.02	0.18 ± 0.03	0.25 ± 0.02	NS	**	NS
Piriform lobe (amygdala)	0.26 ± 0.02	0.29 ± 0.04	0.33 ± 0.02	0.32 ± 0.02	*	NS	NS
Dorsal mesencephalon§	0.43 ± 0.05	0.37 ± 0.12	0.43 ± 0.07	0.49 ± 0.06	NS	NS	NS
Ventral mesencephalon§	0.20 ± 0.02	0.22 ± 0.08	0.31 ± 0.04	0.26 ± 0.04	NS	NS	NS
Pons-medulla§	0.46 ± 0.04	0.33 ± 0.05	0.45 ± 0.04	0.24 ± 0.01	NS	**	NS
Raphe nuclei (mesencephalon)	0.55 ± 0.05	0.46 ± 0.04	0.61 ± 0.03	0.60 ± 0.04	*	NS	NS
Raphe nuclei (pons-medulla)	0.42 ± 0.06	0.56 ± 0.07	0.42 ± 0.04	0.41 ± 0.05	NS	NS	NS

†Results are expressed in μg/g (fresh tissue) (Mean ± SEM). Number of animals is in brackets.
‡A: effects of go-nogo training; B: effects of CDP administration; I: A×B interactions; $*p<0.05$; $**p<0.01$.
§Without raphe nuclei.
¶$p<0.05$; comparison with untrained controls.
‡‡$p<0.05$; comparison with trained controls.

TABLE 3

(Continued)

Experiment 2
Go-Nogo Performance—CDP 10 Days

Untrained		Trained		Factorial* analysis		
Controls (6)	CDP (6)	Controls (5)	CDP (5)	A	B	I
0.90 ± 0.08	0.83 ± 0.02	1.06 ± 0.09	0.94 ± 0.12	NS	NS	NS
0.57 ± 0.04	0.64 ± 0.03	0.60 ± 0.10	0.59 ± 0.05	NS	NS	NS
0.65 ± 0.07	0.76 ± 0.06	0.70 ± 0.06	0.73 ± 0.08	NS	NS	NS
0.44 ± 0.06	0.52 ± 0.04	0.53 ± 0.06	0.54 ± 0.08	NS	NS	NS
0.59 ± 0.05	0.59 ± 0.05	0.57 ± 0.05	0.64 ± 0.04	NS	NS	NS
0.66 ± 0.07	0.81 ± 0.06	0.88 ± 0.09	1.04 ± 0.15	*	NS	NS
0.97 ± 0.07	1.05 ± 0.11	1.09 ± 0.11	1.15 ± 0.07	NS	NS	NS
1.09 ± 0.14	0.98 ± 0.08	1.16 ± 0.12	1.13 ± 0.10	NS	NS	NS
0.72 ± 0.05	0.75 ± 0.06	0.96 ± 0.15	0.84 ± 0.10	NS	NS	NS
0.91 ± 0.08	1.04 ± 0.09	1.14 ± 0.08	1.19 ± 0.04	*	NS	NS
0.66 ± 0.05	0.64 ± 0.05	0.67 ± 0.05	0.86 ± 0.07	*	NS	NS
0.61 ± 0.05	0.69 ± 0.05	0.72 ± 0.04	0.75 ± 0.08	NS	NS	NS

TABLE 4

(Continued)

Experiment 2
Go-Nogo Performance—CDP 10 Days

Untrained		Trained		Factorial* analysis		
Controls (6)	CDP (6)	Controls (5)	CDP (5)	A	B	I
0.40 ± 0.05	0.38 ± 0.04	0.43 ± 0.06	0.37 ± 0.02	NS	NS	NS
0.42 ± 0.03	0.42 ± 0.03	0.61 ± 0.08¶	0.36 ± 0.04‡‡	—	—	*
0.95 ± 0.14	1.26 ± 0.10	1.30 ± 0.16	1.02 ± 0.15	—	—	*
0.34 ± 0.01	0.29 ± 0.02	0.32 ± 0.03	0.26 ± 0.02	NS	* *	NS
2.47 ± 0.26	1.83 ± 0.12	2.42 ± 0.27	2.22 ± 0.30	NS	NS	NS
0.17 ± 0.03	0.16 ± 0.01	0.17 ± 0.03	0.19 ± 0.03	NS	NS	NS
0.28 ± 0.03	0.34 ± 0.02	0.38 ± 0.03	0.36 ± 0.05	NS	NS	NS
0.56 ± 0.06	0.45 ± 0.08	0.41 ± 0.07	0.40 ± 0.12	NS	NS	NS
0.47 ± 0.06	0.42 ± 0.08	0.52 ± 0.05	0.27 ± 0.09	NS	*	NS
0.46 ± 0.04	0.42 ± 0.04	0.44 ± 0.03	0.37 ± 0.08	NS	NS	NS
0.58 ± 0.06	0.54 ± 0.05	0.65 ± 0.06	0.60 ± 0.04	NS	NS	NS
0.50 ± 0.05	0.51 ± 0.04	0.64 ± 0.10	0.52 ± 0.07	NS	NS	NS

TABLE 5

EFFECTS OF CHLORDIAZEPOXIDE (CDP; 0.4 mg/kg/DAY, PER OS) ON THE NORADRENALINE CONTENT IN BRAIN OF
UNTRAINED CATS AND CATS TRAINED ON A GO-NOGO SUCCESSIVE DISCRIMINATION TASK

	Experiment 1						
	Go-Nogo Acquisition—CDP 21–22 Days						
	Untrained		Trained		Factorial‡ analysis		
Structures*	Controls (6)	CDP (6)	Controls (6)	CDP (6)	A	B	I
Frontal cortex	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	NS	NS	NS
Neostriatum	4.94 ± 0.52	4.41 ± 0.49	6.54 ± 0.34	5.68 ± 0.29	*	NS	NS
Septum	0.73 ± 0.18	0.70 ± 0.13	0.66 ± 0.11	0.62 ± 0.04	NS	NS	NS
Piriform lobe (amygdala)	0.16 ± 0.02	0.18 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	NS	NS	NS
Dorsal mesencephalon§	0.09 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.20 ± 0.03	**	**	NS
Ventral mesencephalon§	0.18 ± 0.02	0.19 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	NS	NS	NS

*Results are expressed in μg/g (fresh tissue) (Mean ± SEM). Number of animals is in brackets.
‡A: effects of go-nogo training; B: effects of CDP administration; I: A×B interactions; $*p<0.05$; $**p<0.01$.
§Without raphe nuclei.

hippocampus ($p<0.01$) (Table 3). As shown in Table 4, the NA content was enhanced significantly by the CDP administration in the hypothalamus ($p<0.05$) and hippocampus ($p<0.01$), whereas a significant fall was observed in the pons-medulla ($p<0.01$). In addition, the CDP administration produced a significant increase in the DA level in the dorsal mesencephalon ($p<0.01$) (Table 5).

A significant interaction between the effects of the SD training and those of the CDP administration on the 5-HT concentration was observed in the pons-medulla raphe nuclei, $F(1,19)=4.60$, $p<0.05$ (Table 1). Analysis of simple effects revealed that, in this brain area, the SD training induced a significant increase in the 5-HT level in trained controls ($p<0.05$) (Table 1). A SD training × CDP administration interaction on the 5-HIAA content was also observed in the dorsal mesencephalon, $F(1,18)=5.16$, $p<0.05$ (Table 2), but there was no significant simple effect of training or CDP. As shown in Table 3, such an interaction was also found for the 5-HT:5-HIAA ratio in the dorsal mesencephalon, $F(1,18)=8.26$, $p<0.05$, and pons-medulla raphe nuclei, $F(1,19)=5.38$, $p<0.05$. There was no significant simple effect of training or CDP in the latter brain area, whereas in the former, analysis of simple effects revealed a CDP-induced rise of the 5-HT:5-HIAA ratio in untrained cats ($p<0.05$) (Table 3). In addition, a SD training × CDP administration interaction on the NA level was observed in the thalamus, $F(1,19)=5.83$, $p<0.05$ (Table 4). Analysis of simple effects showed that, in this brain area, the SD training led to a significant increase in the NA concentration in trained controls ($p<0.05$) (Table 4). Moreover, there was no significant interaction between the effects of the SD training and those of the CDP administration on the DA content (Table 5).

EXPERIMENT 2: EFFECTS OF CHLORDIAZEPOXIDE
ON GO-NOGO PERFORMANCE AND BRAIN
BIOGENIC AMINES

METHOD

Animals were assigned to 2 groups of 6 and 2 groups of 5 cats. Group 1 consisted of untrained, manipulated controls, while cats in group 2 included untrained, manipulated cats

treated with CDP (0.4 mg/kg/day, per os) for 10 consecutive days. Subjects in group 3 were trained on SD for 20–22 days. Group 4 included cats trained on SD as group 3 and receiving CDP as group 2.

Subjects were manipulated or trained on SD until they reached the criterion level of 85% correct responses (mean performance on both go and nogo trials) for at least 4 successive sessions. Animals then continued being manipulated or performing the task for 10 more sessions, during which cats in groups 2 and 4 were treated with CDP. The CDP administration began on either day 15, 16 or 17 of training, depending on the subjects. Animals were trained 18 hr after the CDP administration. Training was carried out 6 days a week until the beginning of CDP administration, and then 7 days a week. On the 25th and 26th training days, 8 cats—2 subjects from each of the 4 experimental groups—were killed, and the other 6 animals were killed on the 27th training day.

Behavioral Data

The mean number of trials needed by groups 2 and 4 to reach the acquisition criterion, prior to the onset of CDP administration, was 640±41 and 630±68, respectively, and was not different, $t(8)=0.13$, NS.

Figure 2A illustrates the performance (percentage of correct responses) of controls and CDP treated cats on go and nogo trials, for the 5 days before receiving CDP, and for the 10 consecutive days under CDP administration.

Through the 5 days preceding the onset of CDP administration, the performance on go trials of controls (96.3±2.0%) and CDP treated cats (93.6±5.0%) was not different. There was no group effect, $F(1,8)=0.32$, NS, no days effect, $F(4,32)=0.04$, NS, and no group × days interaction, $F(4,32)=0.97$, NS. On nogo trials, the performance of controls (85.3±2.0%) and CDP treated animals (84.6±2.2%) was not different. In fact, no group effect, $F(1,8)=12$, NS, was observed, whereas a significant days effect, $F(4,32)=11.53$, $p<0.01$, was found. However, there was no group × days interaction, $F(4,32)=2.26$, NS.

Through the 10 days of CDP administration, the performance on go trials of controls (98.3=1.2%) and CDP treated subjects (99.8±0.3%) was not different. No effects of CDP,

TABLE 5

(Continued)

Experiment 2
Go-Nogo Performance—CDP 10 Days

Untrained		Trained		Factorial* analysis		
Controls (6)	CDP (6)	Controls (5)	CDP (5)	A	B	I
0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	NS	NS	NS
6.40 ± 0.76	6.82 ± 0.84	7.37 ± 1.00	7.04 ± 1.19	NS	NS	NS
1.02 ± 0.12	0.75 ± 0.05	0.98 ± 0.12	0.93 ± 0.27	NS	NS	NS
0.16 ± 0.01	0.19 ± 0.03	0.24 ± 0.04	0.17 ± 0.02	—	—	*
0.12 ± 0.01	0.18 ± 0.02	0.15 ± 0.02	0.18 ± 0.05	NS	NS	NS
0.18 ± 0.03	0.20 ± 0.03	0.21 ± 0.05	0.22 ± 0.02	NS	NS	NS

$F_{(1,8)} = 1.85$, NS, no days effect, $F_{(9,72)} = 1.46$, NS, and no CDP administration × days interaction, $F_{(9,72)} = 1.31$, NS, was observed. On nogo trials, the performance (controls $= 87.4 \pm 0.8\%$, CDT treated cats $= 80.2 \pm 5.0\%$) was not affected by CDP. Despite a tendency of CDP treated cats to perform at lower percentages than controls on days 6–10 (Fig. 1A), a difference being mainly due to 3 CDP treated animals, no significant effect of CDP, $F_{(1,8)} = 2.55$, NS, was observed. However, a days effect, $F_{(9,72)} = 3.22$, $p < 0.01$, was noted but there was no CDP administration × days interaction, $F_{(9,72)} = 1.55$, NS.

Figure 2B illustrates the response latencies of correct responses made by controls and CDP treated cats on nogo trials through the 5 days preceding the onset of CDP administration and the 10 days under CDP. Prior to CDP administration, there was no difference between the response latencies of controls (0.98±0.31 sec) and CDP treated animals (1.08±0.32 sec). No group effect, $F_{(1,8)} = 0.06$, NS, no effect of days, $F_{(4,32)} = 0.82$, NS, and no group × days interaction, $F_{(4,32)} = 0.70$, NS, was found. Through the 10 training days under CDP, the response latencies of controls (0.65±0.12 sec) and CDP treated cats (0.54±0.03 sec) were not different. There was no effect of CDP, $F_{(1,8)} = 1.02$, NS, no days effect, $F_{(9,72)} = 1.69$, NS, and no CDP administration × days interaction, $F_{(9,72)} = 0.70$, NS.

Biochemical Data

Tables 1–5 (right part) show the endogenous levels of 5-HT and 5-HIAA, the 5-HT:5-HIAA ratio, and the concentrations of NA and DA, observed in 12 brain areas in the 4 experimental groups. The tables present (1) the neurochemical effects of the SD training (A); (2) the neurochemical effects of the CD administration (B); and (3) the interactions between the effects of the SD training and those of the CDP administration (I).

As shown in Table 1, any significant change in brain levels of 5-HT was observed in cats overtrained on SD. However, the SD performance led to a fall of 5-HIAA content in the neostriatum ($p < 0.05$) and pons-medulla ($p < 0.05$) (Table 2). The 5-HT:5-HIAA ratio was enhanced significantly by the SD performance in the hippocampus ($p < 0.05$),
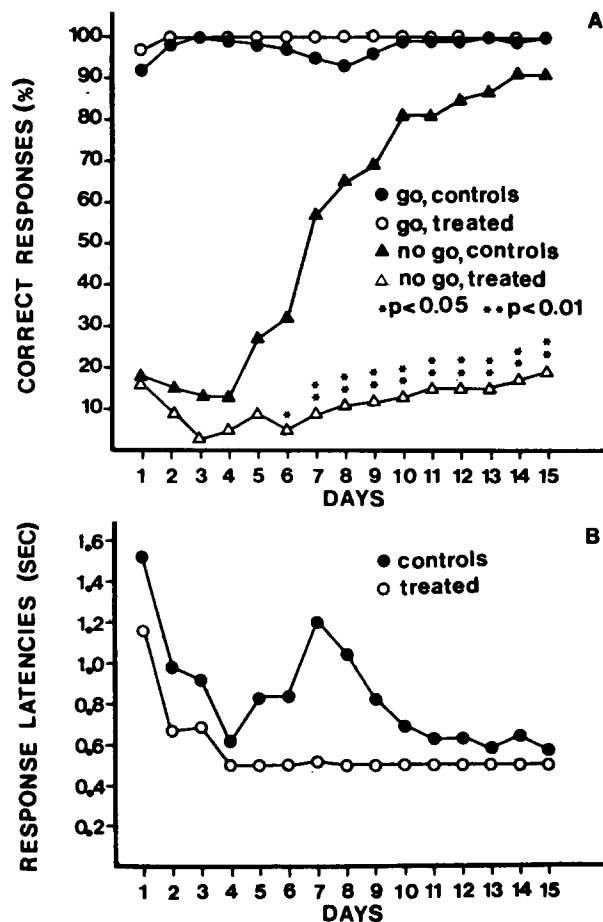

FIG. 2. Effects of chlordiazepoxide (0.4 mg/kg/day) on performance (% of correct responses) on go and nogo trials (A), and on response latencies (sec) on correct go responses (B) of cats trained on a previously learned go-nogo successive discrimination task. Days 1–5 represent the performance of cats at criterion level immediately prior to the onset of CDP administration, and the arrow on day 6 indicates the first day of CDP administration which continued for 10 consecutive days.

pons-medulla ($p<0.05$) and mesencephalon raphe nuclei ($p<0.05$) (Table 3). As shown in Tables 4 and 5, the SD performance did not produce any significant main effect on the brain concentrations of NA and DA.

The 10-day CDP administration induced a significant increase in the 5-HT content in the pons-medulla ($p<0.05$) (Table 1), and significant falls of the 5-HIAA level in the thalamus ($p<0.05$) and mesencephalon raphe nuclei ($p<0.05$) (Table 2). However, the 5-HT:5-HIAA ratio was not significantly affected by the CDP administration (Table 3). In cats treated with CDP, decreases in the NA concentration were observed in the thalamus ($p<0.01$) and ventral mesencephalon ($p<0.05$) (Table 4). As shown in Table 5, the CDP administration did not produce any significant main effect on the brain levels of DA.

A significant interaction between the effects of the SD training and those of the CDP administration on the 5-HT content was observed in the hypothalamus, $F(1,17)=5.04$, $p<0.05$ (Table), but there was no significant simple effect of training or CDP. As shown in Tables 2 and 3, there was no significant SD training $\times$ CDP administration interaction on the 5-HIAA concentration and the 5-HT:5-HIAA ratio. Such an interaction was observed for the NA level in the neostriatum, $F(1,17)=8.25$, $p<0.05$, and septum, $F(1,16)=5.40$, $p<0.05$ (Table 4). Analysis of simple effects revealed that, in the neostriatum, the SD training led to an increase in the NA content in trained controls ($p<0.05$) (Table 4). Moreover, in trained animals, the CDP administration produced a significant fall in NA in the neostriatum ($p<0.05$) (Table 4). In the septum, however, there was no significant simple effect of training or CDP on the NA concentration. As shown in Table 5, a significant SD training $\times$ CDP administration interaction on the DA level was observed in the piriform lobe ($p<0.05$), but there was no significant simple effect of training or CDP in this brain area.

DISCUSSION

The present study has shown that (1) the repeated administration of a clinically relevent dose of CDP produced a strong impairment of the acquisition of an appetitive SD task, but did not significantly affect the performance of cats in a previously learned SD task; (2) the 21–22 days as well as the 10-day CDP administration led to regional modifications of the endogenous levels of biogenic amines in the CNS; (3) the SD acquisition as well as the SD overtraining (performance) were accompanied by selective changes in the contents of brain biogenic amines; and (4) in particular brain areas, interactions were observed between the effects of the SD training and those of the CDP administration on biogenic amines levels.

Behavioral Effects of CDP

The chronic administration of CDP produced a strong impairment of the acquisition of a positively reinforced SD task, in cats trained 18 hr after the daily CDP administration. This effect was related exclusively to a persistent responding on nogo trials. In fact, cats treated with CDP responded to both the positive and negative stimuli throughout the experiment, whereas controls progressively stopped responding on nogo trials.

In contradistinction to this CDP-induced impairment of the SD acquisition, CDP had no significant effect on either the performance or the response latencies of cats performing a previously learned SD task. However, an analysis of indi-vidual data indicates that CDP disturbed the SD performance in some animals. In fact, during the first 5 days of CDP administration (Fig. 2A, days 6–10), the performance in nogo trials of 3 out of the 5 CDP treated cats varied between 16 and 64% depending on the subject. Even though these changes did not reach statistical significance, they suggest that CDP produced, at least in some animals, a disinhibition of responding. Such an effect on inhibition of motor responses has been ascribed to BZP by several investigators using various discrimination tasks in rats [13, 29, 30, 72], pigeons [65], monkeys [23,42] and cats [52]. However, in the present study, the data show that this effect was transient, since during the last 5 days of treatment (Fig. 2A, days 11–15), the performance of all CDP treated cats in nogo trials returned to levels higher than 80% correct responses. Thus, despite a transient and individual impairment of performance, cats treated with CDP were still able to discriminate between the two stimuli, to perform a motor response as well as to refrain from responding. This weak effect of CDP on SD performance might be related to the low dose used, since Schallek *et al.* [52] have shown that the performance of cats in an asymmetric go-nogo successive discrimination task was impaired by 10 mg/kg of CDP but not by a dose of 5 mg/kg.

The present findings have revealed a dissociation between the effects of CDP on acquisition and on performance. Such a dissociation has been previously reported by Barth-alamus *et al.* [2], who have shown that a dose of diazepam of 1.0 mg/kg impaired the acquisition but not the performance of a serial position sequence in pigeons, while both acquisition and performance of this task were disrupted with a dose of 3.0 mg/kg. Moreover, Thompson [62] have reported, on the one hand, that CDP (10 and 20 mg/kg) impaired acquisition more severely than performance in pigeons trained on a four-response chain task, and, on the other hand, that tolerance to these CDP-induced effects developed more rapidly under the performance condition than under the learning condition.

Numerous studies, using behavioral tasks involving a punishment, a suppression of responding, an extinction of motor responses or a nonreward component, have ascribed to the BZP a behavioral disinhibition action [6]. Moreover, several authors have reported that BZP impair the acquisition or the performance of various discrimination tasks in rats [13, 20, 29, 30], pigeons [2, 50, 62], monkeys [23,42] and cats [52]. In most of these investigations, it has been suggested that BZP induce a disinhibition of responding. In contrast to these studies, there has been increasing evidence in recent years that BZP may also exert an action on memory and learning processes. In fact, experiments done in animals and humans have suggested that BZP may affect these processes by disrupting information processing at the encoding stage [50], by interfering with the registration or events following it [56], or by impairing acquisition of new information without impairing recall processes [15, 25, 36].

In the light of the above reports, the present findings might reflect an effect of CDP either on requisitional processes or on behavioral inhibition mechanisms. On the one hand, cats treated with CDP during the performance of an already learned SD task were still able to refrain from responding in nogo trials, whereas animals treated with the same dose during acquisition did not learn the SD task and did not improve this performance throughout the 15 training days. These observations thus raise the possibility that CDP might interfere with mechanisms related to acquisition or

registration processes. On the other hand, the differential effects of CDP on acquisition and performance might be due also to differences between these two states of training, in respect to (1) the level of response inhibition, and (2) the level of punishment effects of nonreinforcement in nogo trials. Since the SD acquisition requires higher levels of response inhibition and involves stronger punishment effects of non-reinforcement in nogo trials than the SD performance, it is thus possible that the disinhibitory action of CDP impairs more severely the acquisition than the performance condition. Although further investigations are needed to dissociate the effects of BZP on memory and learning, and on behavioral inhibition, the present experiments have demonstrated that a clinically relevant dose of CDP does not induce a general disruption of behavioral inhibition mechanisms, but severely impairs the acquisition of a positively reinforced SD task.

Neurochemical Effects of CDP

The long-term administration of a clinically relevant dose of CDP produced regional modifications of the endogenous levels of 5-HT, 5-HIAA, NA and DA in cat brain. The present findings indicate that these neurochemical changes were long-lasting, as animals were killed 18 hr after the last CDP administration.

Most of the previous works which have investigated the effects of BZP on brain biogenic amines have used large doses and have observed the effects of these drugs in the whole brain or in a few brain areas. In contrast to these studies, the present biochemical data reveal that the effects of a clinically relevant dose of CDP on monoamines are produced selectively in particular cerebral structures, thus dissociating them from the rest of brain.

It has been reported by several authors that BZP reduce the turnover and utilization of 5-HT [1, 7, 31, 37, 43, 45, 47, 69], NA [8, 14, 45, 46, 47, 61] and DA [8, 14, 32, 45, 46, 61] in the CNS. In the present study, the increased 5-HT levels, the decreased 5-HIAA content and the enhanced 5-HT:5-HIAA ratio which were induced by CDP in many brain areas, are in agreement with those reports and suggest a localized decrease in serotoninergic activity. Moreover, the present findings show a neuroanatomical dissociation of the effects of CDP on brain NA. In fact, the NA concentration was either enhanced or lowered by the CDP administration, depending on the cerebral structure and the treatment duration. Even though the metabolites of NA were not assayed, the CDP-induced increases in the NA level may reflect an accumulation resulting from a reduced release of this amine. This interpretation is supported by the findings of Rastogi et al. [46], who have shown that diazepam produced a rise in the NA content and a fall of the 4-hydroxy-3-methoxy-phenylglycol (MHPG) concentration in the CNS. On the other hand, the CDP-induced decreases in the NA level may reflect an enhanced noradrenergic activity. Although the neuropharmacological mechanisms underlying these decreases are unknown, the BZP derivative and the dose used may be important factors. In fact, to our knowledge, CDP is the only BZP derivative which has been reported to induce a decrease in the brain NA content [12,64]. In this respect, Fenessy and Lee [12] have shown that among 6 BZP derivatives administered at a motor ED_{50} dose, CDP was the only one to produce a decrease in the whole brain NA level, while the other drugs induced an increase or no effect. Furthermore, Vachon and Roberge [67] have recently ob-

served that a 7-day CDP administration with doses of 0.4 and 10 mg/kg produced either a rise or a decline in the NA content depending on the cerebral areas, while only increases were found with a dose of 20 mg/kg. In addition, as reported by Vachon and Roberge [67], the present findings show that a low dose of CDP produces few effects on brain contents of DA. The only significant change observed in the DA level was an increase in the dorsal mesencephalon in cats receiving CDP for 21–22 days. This rise may reflect an accumulation of the amine, resulting from a selective reduction in dopaminergic activity in this brain area.

Previous investigations have shown, in mice or rats, that the changes produced by BZP on the brain 5-HT and 5-HIAA contents may be either maintained [47] or returned to control levels [31,69] over a long period of administration. In contrast to these studies, the present data have shown that more numerous changes in the 5-HT and 5-HIAA concentrations as well as in the 5-HT:5-HIAA ratio, were found after the 21–22 day than the 10-day CDP administration. These effects might be due to an accumulation of either CDP or its metabolites within the CNS. In this respect, it has been reported, in humans receiving a daily dose of CDP of 0.5–0.8 mg/kg, that the half-life of CDP was of 10–18 hr, while the half-lives of demoxepam and diomethyldemoxepam, two CDP metabolites, were longer than 35 hr [3,19]. Furthermore, Randall and Kappel [44] have observed that the metabolites of CDP are pharmacologically active. On the other hand, long-term modification in uptake, synthesis and degradation of 5-HT might also account for the time-increasing effects of CDP on indoleamines. In fact, Agarwal et al. [1] have shown that a 22-day diazepam treatment produced an enhanced tryptophan hydroxylase activity in the midbrain and a lowered monoamine oxidase (MAO) activity in the whole brain. In addition, Rastogi et al. [47] have reported that a 22-day treatment with diazepam, but not a single administration, induced an increase in 5-HT synthesis, 5-HT uptake and tryptophan levels in the whole brain synaptosomes.

It has been postulated that BZP exert their effects on behavior by reducing the utilization of 5-HT and NA in the CNS [14, 47, 54, 60, 69]. Moreover, neurophysiological studies have shown that cerebral neuronal activity was particularly affected by these drugs in brainstem and limbic structures [16, 18, 21, 51]. In agreement with these studies, the present results have revealed that a clinically relevant dose of CDP produced neurochemical changes that were localized in the diencephalon and in particular regions of the brainstem and limbic system. As already discussed, the effects of CDP on the SD acquisition may be due to an action of this drug either on acquisition or registration, on behavioral inhibition mechanisms, or on the punishing effects produced by non-reinforcement in nogo trials. Therefore, the CDP-induced neurochemical changes, and the CNS areas in which these effects were observed, might reflect a CDP-induced impairment of various brain mechanisms. For instance, the limbic system has been implicated in behavioral inhibition, in reward and punishment, as well as in memory and learning [27]. However, even though further investigations are needed to precise the specific behavioral mechanisms which might be related to these regional biochemical modifications, the present findings emphasize a possible involvement of selective diencephalic, brainstem and limbic monoaminergic neurons in the SD acquisition impairment produced by the CDP administration and hence in the behavioral effects of this drug.

Neurochemical Effects of Training

The SD acquisition, as well as the SD overtraining (performance), were accompanied by selective changes in the endogenous levels of 5-HT, 5-HIAA, NA and DA in the CNS. It is unlikely that these biochemical modifications were related to unspecific variables such as stress, manipulation or food conditions, since controls were submitted to the same experimental conditions (without training) as trained animals. These findings are thus in agreement with previous studies which have shown that operant behavior involves modifications in the metabolism of monoamines in particular brain areas [9, 11, 24, 35, 68].

The neurochemical changes found in cats killed after the SD acquisition were different from those observed in animals overtrained on this task. It is unlikely that these differences were due to the duration of training, since it has been shown that different operant schedules led to different effects on brain monoamines in normal rats trained for the same number of days [9,53]. Moreover, Everett and Roberge [11], using the same apparatus as in the present study, have observed no modification in brain biogenic levels in cats maintained for 8 days on the shaping period during which they were trained to perform the motor response for food reinforcement but were not submitted to discrimination learning. The present results thus indicate that different behavioral states are associated with different neurochemical changes and different neuroanatomical structures, as reported in previous investigations [9,35]. Moreover, it is interesting to observe that the SD acquisition was accompanied by more numerous changes in monoamines levels than the SD overtraining. These findings are consistent with the view that acquisition involves more complex mechanisms than the performance of an already learned task. In this regard, one might expect that a given neurochemical change observed after overtraining would be found also after acquisition, since the latter requires behavioral processes necessary to learn the task as well as to maintain the performance. However, the SD acquisition and the SD overtraining led to quite different biochemical modifications in the CNS. Such a finding has also been reported by Vachon and Roberge (submitted for publication), who have shown that the neurochemical changes observed in normal cats trained for 5 days on a 2-choice successive discrimination task were different from those found by Everett and Roberge [11] in cats trained on the same task but killed after they had reached the acquisition criterion. Although further investigations are needed to elucidate the mechanisms underlying these effects. The present experiments have shown a differentiation in the changes in brain biogenic amines metabolism and the selective monoaminergic pathways implicated respectively in the acquisition and the performance of the SD task.

The neurochemical changes associated with the SD training may reflect various behavioral processes, since the cerebral monoaminergic systems have been suggested to be implicated in memory and learning [26], as well as in other aspects of behavior that are involved in the SD training. Brain 5-HT has been postulated to be implicated in arousal [41], behavioral inhibition [17, 63, 70] and punishment [58,66]. The cerebral noradrenergic systems have been also suggested to be involved in arousal [41], attention [40] and reward [59]. Moreover, a role has been ascribed to DA in motor organization and motivation [28].

Neurochemical changes observed after training were observed in many brain areas mediating behavioral processes in which the monoamines have been reported to be involved. For instance, the frontal cortex and the neostriatum has been implicated in inhibition mechanisms [58] and motor organization [28], respectively. The amygdala has been reported to be involved in motivation, arousal and behavioral inhibition [27]. The brainstem, which participates also in arousal mechanisms [72], includes monoaminergic cell bodies, hence possible interactions among 5-HT, NA and DA.

Although several mechanisms remain to be elucidated, the present findings emphasize that training is accompanied by selective changes in the metabolism of biogenic amines, thus suggesting their involvement in operant behavior. However, it is likely that other neurotransmitters such as acetylcholine, γ-aminobutyric acid (GABA), amino acids as well as peptides may be implicated.

SD Training × CDP Administration Interactions

In particular brain areas, interactions were observed between the effects of the SD training and those of the CDP administration, on levels of biogenic amines, thus suggesting that the behavioral state of animals may interfere with the neurochemical effects of CDP.

During the past few years, numerous studies have given increasing evidence that GABA mediates the primary action of BZP [48,51]. In this respect, Costa and Guidotti [4] have suggested that these drugs may enhance indirectly the action of GABA on post-synaptic receptors. Moreover, it has been postulated that GABA-ergic mechanisms are involved in the effects of BZP on serotonergic [49,61] noradrenergic [14] and dopaminergic [33] neurons. Since it has been shown that operant behavior may be accompanied by regional changes in brain GABA levels [9], the localized SD training CDP administration interactions that were observed in the present study may represent regional training-associated modifications of GABA-ergic activity. Such a change in the GABA levels present at the GABA receptors site would thus influence indirectly the effects of CDP on monoamines. This hypothesis, however, remains to be verified.

CONCLUSION

The present study has shown that (1) the acquisition but not the performance of a SD task was strongly impaired in cats by a clinically relevant dose of CDP; this impairment may be due to an action of CDP on behavioral inhibition or punishment mechanisms, but also to a CDP-induced disruption of acquisition or registration processes; (2) the chronic administration of CDP produced regional and long-lasting modifications in endogenous levels of biogenic amines in the CNS, suggesting the involvement of particular monoaminergic neurons in the behavioral effects of CDP; (3) more numerous changes in the indoleamines contents were observed after the 21–22 day than after the 10-day CDP administration, thus suggesting a possible accumulation of CDP or its metabolites within the CNS; (4) the SD acquisition, as well as the SD overtraining, were accompanied by selective modifications in brain monoamine levels. Thus emphasizing that biogenic amines are implicated in operant behavior; and (5) in particular brain areas, significant interactions were observed between the effects of the SD training and those of the CDP administration on concentrations of monoamines, indicating that the behavioral state may interfere with the neurochemical effects of CDP.

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