Pharmacology Biochemistry & Behavior, Vol. 12, pp. 767–771. Printed in the U.S.A.

Effects of Diazepam-Infrasounds Combination on Locomotor Activity and Avoidance Behaviour of Rats

C. SPYRAKI, Z. PAPADOPOULOU, B. ZIS AND D. VARONOS

Laboratory of Experimental Pharmacology, Medical School, University of Athens
Goudi 609-Athens, Greece

Received 27 October 1979

SPYRAKI, C., Z. PAPADOPOULOU, B. ZIS AND D. VARONOS. *Effects of diazepam-infrasounds combination on locomotor activity and avoidance behaviour of rats.* PHARMAC. BIOCHEM. BEHAV. 12(5) 767–771, 1980.—The locomotor activity, the two-way conditioned avoidance response, the norepinephrine and dopamine brain levels, were simultaneously studied in rats, under the influence of Diazepam alone or in combination with infrasound environment. Diazepam decreased the spontaneous activity and facilitated the retention of an avoidance behaviour of rats subjected to four shuttle box sessions. These effects were reversed when Diazepam was administered to rats exposed to infrasound environment. The norepinephrine and dopamine levels, determined in whole brain homogenate, were slightly affected in both cases.

Infrasound environment Diazepam Avoidance learning Locomotor activity Brain catecholamine levels

THE effects of benzodiazepines on animal behaviour have been studied in a number of investigations, with conflicting results. Several reports support the finding that administration of diazepam significantly decreases the spontaneous locomotor activity [1,10] while other data indicate that benzodiazepines enhance hyperkinetic behaviour induced by amphetamine [7]. Performance in the shuttle-box is facilitated by benzodiazepines, especially during early acquisition [11]. When the drug is given, after training is complete, disruption of performance may be seen [5,11].

Biochemical and histochemical studies indicate that benzodiazepines decrease the turnover of catecholamines in the brain. It thus seems reasonable to speculate that the decreased turnover of these substances may be at least partly responsible for the punishment-lessening effects of tranquilizers [13]. On the other hand in our previous studies we reported that infrasounds produce a decrease in the exploratory activity of rats, suppression of locomotor activity [8], a sleep-like state, an inhibition of the acquisition and retention of a conditioned avoidance response [9], and a decrease of brain norepinephrine levels [12].

In order to extend the investigation on the effects of infrasounds on these particular aspects of animal behaviour, it seems suitable to consider also combinations of the infrasound environment with tranquilizing agents, since these substances are widely used in everyday activities (cars, factories where establishment of an infrasound environment is possible [2]) and in research concerning the central nervous system.

For this purpose, in the present study, infrasound environment has been combined with diazepam, a minor tranquilizer drug often tested in avoidance situations.

In the shuttle box, in particular the effect of the combination on the consolidation processes was studied by injecting the drug after each training session and by letting the animal for an hour in an infrasound environment. It is in fact known [6] that the consolidation processes may be influenced by the post-trial administration of drugs; drugs that improve performance may be regarded as enhancing memory, while drugs that impair performance may be regarded as disrupting memory.

Finally, the effects of infrasound environment plus diazepam on locomotor activity and on brain catecholamines levels were investigated in rats. The effects of passing from drug treatment or infrasound environment to saline solution and vice versa have also been considered.

METHOD

EXPERIMENT 1: AVOIDANCE BEHAVIOUR

Animals

A total of 40 adult male Wistar rats (200–300 g) were used. They were divided into four groups, each receiving a different treatment: (a) saline (control groups); (b) diazepam (5mg/kg) (Valium-Hoffman La Roche); (c) infrasound environment (16 Hz, 124 dB). (d) diazepam plus infrasound environment (ISE).

Apparatus and Procedure

The apparatus used for the study of the conditioned avoidance behaviour was a two-way shuttle box described previously [9]. The infrasound generation system, consisting

of a low voltage supply and the infrasound chamber was described elsewhere [8].

The training procedure involved 60 trials on one day, and 20 to 60 trials in the same box, one, three and six days later. Each animal was placed in the standard avoidance conditioning apparatus for 30 min. An initial 5 min adaptation period was allowed to elapse.

The conditioned stimulus (CS light) preceded by 2.5 sec the onset of the unconditioned stimulus (US electric shock) and overlapped it for 4.2 sec. By this procedure the light was present in the compartment for 6.7 sec (2.5 sec alone and 4.2 sec together with the US). A rest period of 23.3 sec followed and the cycle began again. The unconditioned stimulus was an electric shock (45 V) applied to the grid floor. A conditioned response was recorded when the animal avoided the US by running into the other compartment within 3 sec after the onset of the CS. If animals failed to avoid the shock, they could escape it by crossing during the US (4.2 sec).

The following parameters were utilized for analysis of these data:

(1) Rate of performance (RP); the mean time in sec of the 60 efforts. (2) Errors scores (ES); the number of unsuccessful efforts in avoidance response. (3) Conditioned stimulus latency (CSL; the mean time in sec of the avoidance responses. (4) Unconditioned stimulus latency (USL); the mean time in sec of escape responses.

We have selected only rats who had received criterion (15 consecutive avoidance responses) on the first session. This criterion was adopted in order to ensure a homogenous population in which to investigate post-trial treatment effects, as well as to avoid any eventual influence of a low learning ability on later performance [4].

The test was performed between 2 and 5 p.m. in a room isolated from external noise.

The treatments consisted of post-trial injections of saline (group a), diazepam, 5 mg/kg (group b and d). Rats of the group c and d were subjected to infrasound environment (16 Hz, 124 dB) for 1 hr immediately after each training session. All injections were given in a volume of 0.5 ml, 2 min after the last trial of each session.

EXPERIMENT 2: LOCOMOTOR ACTIVITY

The spontaneous locomotor activity was quantitated with the use of an Activity meter (Model 7400, Ugo Basile, Milano, Italy) described previously [8]. This was done under identical conditions at the same time of the day in a light and temperature-controlled, sound proof room. Control rats as well as those given diazepam or exposed to infrasounds were removed in separate plastic cages resting on an activity meter, coupled to a printing counter, for 5 min of exploration before actual recording was carried out over a 30 min session. The data were printed every 5 min, so that the 30-min session was divided into six 5-min intervals. Rats were divided into 4 groups as in Experiment 1, each containing 10 animals. They were dosed daily for 4 days with either diazepam-5 mg/kg—(group b and d), or saline (group a) and exposed for 1 hr for 4 consecutive days to infrasounds (group c and d). All dosing was IP in volumes of 0.5 ml. The spontaneous locomotor activity was estimated 24 hr after the last injection of saline or diazepam or after the last exposure to infrasounds.

The statistical analysis of the data was performed using the *t*-test method.

EXPERIMENT 3: ESTIMATION OF NOREPINEPHRINE (NE) AND DOPAMINE (DA)

The experiment was carried out with 40 male Wistar rats. In this experiment as in the first and the second one the same four groups were formed. Animals were treated with saline (group a) or with diazepam-20 mg/kg divided in four doses administered in consecutive days—(group b and d) or exposed to 1 hr daily session for 4 days to infrasounds.

Twenty-four hours after the treatment was over, rats were sacrificed by decapitation. Whole brains were removed and the cerebellum was discarded. The brains were weighed and frozen on dry ice. NE and DA were estimated fluorometrically by the method of Anton and Sayre [3] with slight modifications described previously [12]. The decay time of NE and DA was controlled in a series of experiments and the chosen storage time (5 days) was the same for all the groups. The statistical analysis of the data was performed using the *t*-test method.

RESULTS

EXPERIMENT 1: AVOIDANCE BEHAVIOUR

The retention of an acquired avoidance behaviour (1st session) under the influence of infrasounds and diazepam, given alone or in combination with infrasound exposure, was evaluated during three post-treatment sessions. Rate performance, mean percent wrong responses, rapidity of avoidance and rapidity of escape, exhibited by rats receiving the same treatment, were calculated and reported in Figs. 1, 2, 3, 4.

A trend analysis for the retention of the acquired avoidance behaviour revealed differences between groups and between sessions. Control rats (saline) exhibited a high performance and a decrease of wrong responses which was more than 50% in the last session. Conditioned stimulus latency was not affected in the course of the sessions, but the unconditioned stimulus latency was decreased in all post-treatment sessions, which reflects an increase of the rapidity of escape.

The diazepam treated group followed the control one, in all the reported parameters. So it may be considered that diazepam did not interfere in the retention of the acquired avoidance behaviour at the dose level tested.

Infrasound exposure after each session, did not provoke changes between sessions for all the calculated parameters. The achieved level in rate performance, errors score, conditioned and unconditioned stimulus latency in all post-treatment sessions was the same as that achieved by the naïve rats (1st session). This finding compared to the results of the control group may reflect an inhibitory effect on the retention of the acquired avoidance behaviour. Rats treated with diazepam and exposed to infrasounds after each session exhibited poor performance as can be seen by the results of the three post-treatment sessions. This inhibitory effect of the diazepam-infrasound combination could be considered as an amnesic one, because it provokes loss of a traced information.

Comparison between groups demonstrated that control rats and diazepam treated rats (receiving saline or diazepam after each session, respectively) showed an increment of avoidance response during the post-treatment sessions, while an avoidance decrement always occurred, when rats were exposed to infrasounds. This effect, slight in the two first post-treatment sessions, was more evident and statisti-

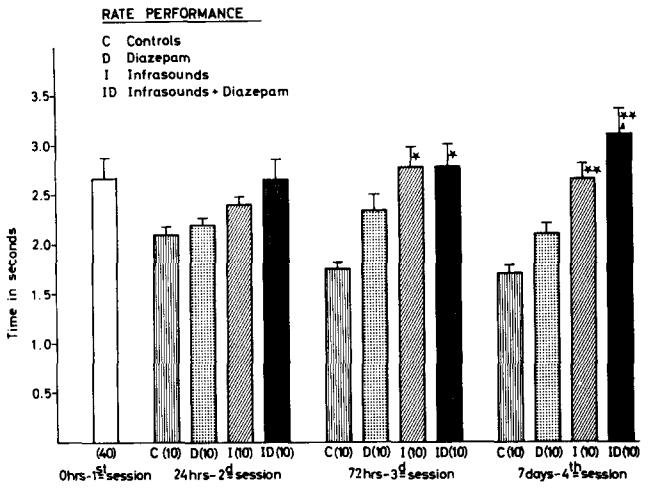

FIG. 1. The effect of diazepam, ISE and diazepam+ISE on rate performance of rats during 4 sessions. Significantly different ★★ ($p<0.01$), ★ ($p<0.05$) from controls, ▲ ($p<0.05$) from diazepam.

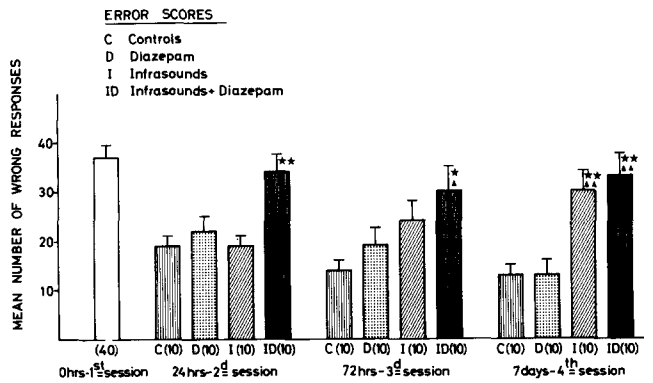

FIG. 2. The effect of diazepam, ISE and diazepam+ISE on error score of rats during 4 sessions. (vertical bars: means ± SE). Numbers of animals are in parentheses. Significantly different ★★ ($p<0.01$), ★ ($p<0.05$) from controls, ▲▲ ($p<0.01$), ▲ ($p<0.05$) from diazepam.

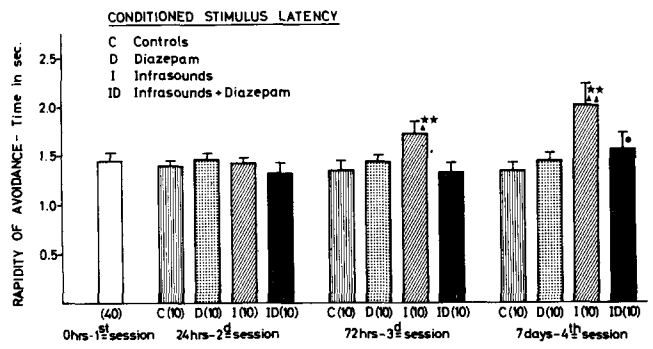

FIG. 3. The effect of diazepam, ISE and diazepam+ISE on conditioned stimulus latency of rats during 4 sessions. (vertical bars: means ± SE). Numbers of animals are in parentheses. Significantly different ★★ ($p<0.01$) from controls; ▲▲ ($p<0.01$), ▲ ($p<0.05$) from diazepam, ● ($p<0.01$) from ISE.

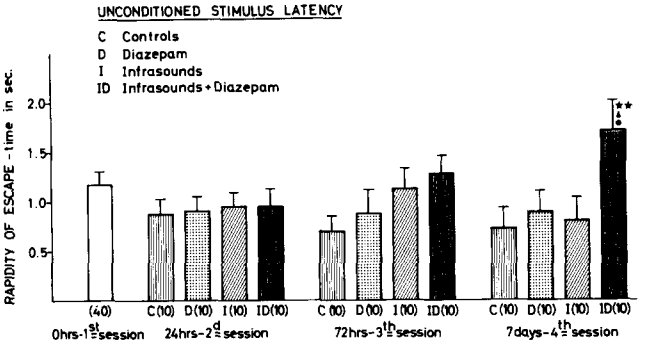

FIG. 4. The effect of diazepam, ISE and diazepam+ISE on unconditioned stimulus latency of rats during 4 sessions. (vertical bars: means ± SE). Numbers of animals are in parentheses. Significantly different ★★ ($p<0.01$) from controls, ▲ ($p<0.05$) from diazepam, ● ($p<0.05$) from ISE.

cally significant in the fourth session. Also it was stronger and more pronounced in the combination of the ISE with diazepam.

EXPERIMENT 2: LOCOMOTOR ACTIVITY

The results are summarized in Fig. 5. Analysis of the data revealed the following changes in the treated groups, when compared to controls:

Administration of diazepam significantly decreased the spontaneous locomotor activity from the control values. Repeated infrasounds exposure did not alter the activity level of the experimental animals. In contrast to diazepam given alone, combination with infrasounds increased the locomotor activity. It may be noted that the change in locomotor performance, induced by diazepam-infrasounds combination was statistically non-significant. The analysis showed significant differences between the diazepam treated group and diazepam-infrasounds combination treated one. This finding demonstrates an antagonizing effect between diazepam and infrasounds on the spontaneous locomotor activity.

EXPERIMENT 3: NA AND DA LEVELS

Results presented in Fig. 6 demonstrate that administration of diazepam alone produced no appreciable effect on the NE or DA levels of whole brain. In agreement with previous experiments [12] the infrasounds exposed rats displayed decrease of whole brain NE levels and of DA ones. However combination of infrasounds exposure with diazepam administration provoked a significant increase of brain NE levels and a more pronounced decrease of DA ones.

DISCUSSION

The results of the present study will be discussed by considering, on one hand, the effects of diazepam given alone and of infrasounds exposure separately, and, on the other hand, the effects of the combination of diazepam with infrasounds. Diazepam, administered alone (20 mg/kg divided in 4 doses, each one administered after each session) improved the avoidance behaviour as compared to the first session (naïve animals). This improvement was similar to

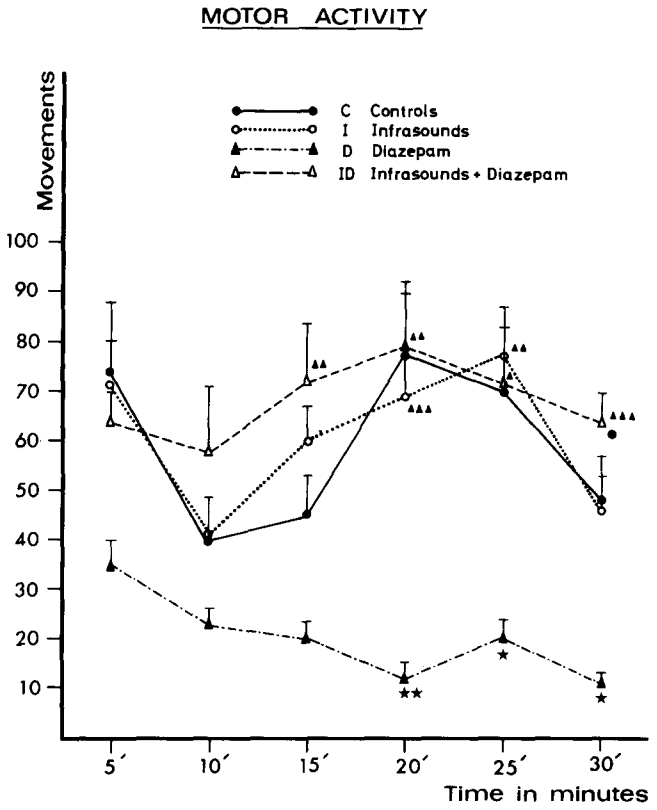

FIG. 5. The effect of diazepam, ISE and diazepam + ISE on locomotor activity of rats. (vertical bars: means ± SE). Significantly different ★★ ($p<0.01$), ★ ($p<0.05$) from controls, ▲▲▲ ($p<0.001$), ▲▲ ($p<0.01$), ▲ ($p<0.05$) from diazepam.

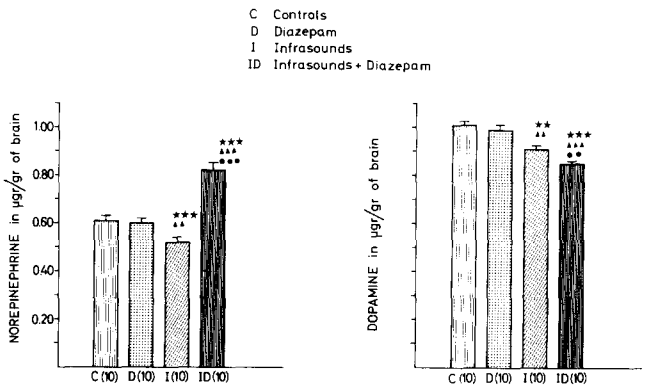

FIG. 6. The effects of diazepam, ISE, diazepam + ISE on NE and DA levels of whole brain of rats. (vertical bars: means ± SE). Numbers of animals are in parentheses. Significantly different ★★★ ($p<0.001$), ★★ ($p<0.01$) from controls, ▲▲▲ ($p<0.001$), ▲▲ ($p<0.01$) from diazepam, ●●● ($p<0.001$), ●● ($p<0.01$) from ISE.

that achieved by control rats. Furthermore diazepam at the same dose level decreased the spontaneous locomotor activity as compared to the controls. The levels of catecholamines were slightly and insignificantly decreased.

Repeated infrasounds exposure provoked an avoidance depression, a slight increase of spontaneous locomotor activity and a decrease of NE and DA whole brain levels of the rats. The results obtained with diazepam agree with previous findings showing avoidance facilitation, depressed locomotion and decrement of NE levels.

As concerns infrasounds exposure, previous studies have demonstrated an inhibitory effect on memory process and a decrease of NE whole brain levels, but also a significant decrease of locomotor activity. In the present study, the finding that no appreciable changes in locomotor activity induced by infrasounds is not surprising. In this respect, it must be noted that the present results are based on different procedures (repeated infrasounds exposure and estimation of activity in a normal environment) from those usually used in previous studies.

As regards the combination of diazepam with infrasounds, a more appropriate statistical analysis could have indicated whether the effects of such combination must be considered "additive" or "interactive".

However, even a simple analysis of the results allows a differentiation between the diazepam-infrasounds combination. When infrasounds combined with diazepam maintain their depressant action on avoidance responding, their stimulant effect on locomotor activity and their ability to decrease the DA whole brain levels.

These effects appear more evident, when infrasounds are combined with diazepam. The effects of the combinations on NE whole brain levels appear on the other hand quite different, as infrasounds exposure decreased NE levels and infrasounds-diazepam combination increased them.

It is difficult, at present, to infer clinical implications from the present findings. These results provide evidence of the ability of an infrasound environment in combination with centrally acting drugs, to produce in laboratory animals behavioural and biochemical changes. These unexpected effects provoked by the ISE influence should be considered when the pharmacological profile of a drug is studied.

REFERENCES

1. Ahtee, L. and E. Shillito. The effect of benzodiazepines and atropine on exploratory behaviour and motor activity of mice. *Br. J. Pharmac.* **40:** 361–371, 1970.
2. Anastassiadis, A., C. Panayotopoulos and C. Thanassoulas. Infrasonic resonances observed in small passenger cars travelling on motorways. *J. Sound Vibrat.* **29:** 257–259, 1973.
3. Anton, A. and D. Sayre. A study of the factors affecting the aluminum oxide Trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.* **138:** 360, 1962.
4. Bovet, D., F. Bovet-Nitti and A. Oliverio. Genetic aspect of learning and memory in mice. *Science* **163:** 139–149, 1969.
5. Chisholm, D. C. and J. W. Moore. Effects of chlordiazepoxide on discriminative fear conditioning and shuttle avoidance performance in the rabbit. *Psychopharmacologia* **18:** 162–171, 1970.
6. McGaugh, J. L. Time dependent processes in memory storage. *Science* **134:**328–342, 1966.

7. Montuori, E., H. A. Gonzàlez and E. E. Ceñal. Estudio farmacologico de una mezela de clorometifenilbenzodiazepinona y clorhidrato de clorometaminodiazepoxido. *Revta Soc. argent. Biol.* **45:** 88–98, 1969.
8. Petounis, A., Ch. Spyraki and D. Varonos. Infrasound effects upon activity levels of rats. *Physiol. Behav.* **18:** 153–157, 1977.
9. Petounis, A., Ch. Spyraki and D. Varonos. Effects of infrasounds on the conditioned avoidance response. *Physiol. Behav.* **18:** 147–152, 1977.
10. Rastogi, B. R., R. A. Agarwal, Y. D. Lapierre and L. R. Singhal. Effects of acute diazepam and clobazam on spontaneous locomotor activity and central amine metabolism in rats. *Eur. J. Pharmac.* **43:** 91–98, 1977.
11. Sachs, E., M. Weingarten and N. W. Klein. Effects of chlordiazepoxide on the acquisition of avoidance learning and its transfer to the normal state and other drug conditions. *Psychopharmacologia* **9:** 17–30, 1966.
12. Spyraki, Ch., Z. Papadopoulou-Daïfoti and A. Petounis. Norepinephrine levels in rat brain after infrasound exposure. *Physiol. Behav.* **21:** 447–448, 1978.
13. Stein, L., D. Wise and J. Belluzzi. Neuropharmacology of reward and punishment. In: *Handbook of Psychopharmacology, Vol. 8,* edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York/London: Plenum Press, 1977, p. 25.