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Effects of Apomorphine and Diazepam on a Quickly Learned Conditioned Suppression in Rats

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KAMEYAMA, T. AND M. NAGASAKA. *Effects of apomorphine and diazepam on a quickly learned conditioned suppression in rats.* PHARMAC. BIOCHEM. BEHAV. 17(1) 59–63, 1982.—Electric shock experienced rats exhibited a marked suppression of motor activity when placed in the same environment in which the animal received shocks. At 24 hr after electrical shocks, the shock frequencies of 1/10 Hz to 1/180 Hz equally produced a marked suppression of motility. However, electric shock experienced rats exhibited a similar degree of locomotor activity that was seen with the non-shocked rats, when the motility of the animals was measured by using Animex system at 24 hr after electrical shocks. Therefore, this suppression of motility was found to be a consistent response and to be a conditioned response to the environment. Diazepam (1, 2.5 or 5 mg/kg, IP) did not show any effect on the conditioned suppression. Apomorphine-HCl (0.1, 0.5 or 1 mg/kg, IP) caused attenuation of the conditioned suppression in relation to dose, but did not increase motor activity of the non-shocked rats. Thus, conditioned suppression caused by a quick conditioning technique may not be associated with anxiety.

Apomorphine Conditioned suppression Diazepam Anxiety Rats

THE emotional responses induced by stresses in experimental animals have been considered to be useful for studying human emotionality [23], although a difference between species in the course of development of the central nervous system is quite significant. Studies on anxiety have used such techniques as those of an open-field [13, 14, 21], social interaction anxiety test [5,7], and fighting behavior [32]. Using these techniques, emotional responses were induced by changing the behavioral situation of animals. In a conditioning paradigm, methods used include: a conditioned emotional response with lever pressing as an indicator of anxiety [3, 4, 16, 17], behavioral conflict [6, 9, 10, 11, 12], autonomic responses, i.e., the change in heart beat [8, 15, 18, 20, 22, 25], and self-stimulation behavior [24,31]. The conditioning techniques, however, proved to be inconvenient for experiments with a large number of animals, and require many hours to produce stable operant baselines. Thus, a simple and stable method to demonstrate the drug action has been developed. For example, Babbini *et al.* [2], reported that rats exhibited a marked reduction of motor activity when placed in an environment in which the animals received electrical shocks, and that this behavioral reduction is to be taken as an indication of a conditioned emotional response. Additionally, morphine diminished such behavioral suppression by means of reducing the pain-anticipatory anxiety.

Diazepam, an anti-anxiety drug, may diminish the behavioral suppression caused by a quick conditioning technique as described above, as well as conditioned emotional response [1]. However, qualitative aspects of a quick conditioning technique have not been reported. Thus, the purpose of the present study is not only to determine whether a quick conditioning technique provides a stable suppression of motility of rats and whether the response depends on the environment in which mice received electric shocks, but also to investigate the mode of apomorphine and diazepam on a conditioned suppression.

METHOD

Male Wistar rats, weighing initially 280–330 grams, were housed in groups of 5 in a constant environment (22±0.5°C, 50±5%) for about 3 weeks and fed food and water, ad lib. Room lights were turned off between 8:00 a.m. and 8:00 p.m. Prior to the experiment animals were handled for 7 days.

The experiments were carried out in a transparent acrylic rectangular cage (24.5×31×30 cm) with a grid floor. The apparatus was located in a sound attenuated room and lit with a 20-W bulb. Behavioral testing was conducted between 6:00 p.m. and 9:00 p.m., and was monitored through a TV system. On day 1, an animal was left in the cage for 9 min and received brief shocks (100 V, DC) for 200 msec through an isolated stimulator (Nihon Kohden, Tokyo, Japan). The current resistance, where an animal was placed, varied between 200 and 500 kΩ. Therefore, an animal received electric shock in range of 0.2–0.5 mA. On day 2, the animal was placed again in the same cage of day 1, but electric shocks were not delivered in this time and its motility changes were recorded automatically with electronic digital counters for

three consecutive 3 min periods by means of infrared cell sensors placed on walls (Opto-varimex, Columbus Instruments, OH). A total of 20 sensors, 2.5 cm apart, was equally mounted 2.5 cm above the grid floor. Control animals received the same procedure as shocked animals except for the non-delivery of shocks on day 1 (non-shocked animals).

On the study of the shock frequency, a group of 7 rats received the shock frequencies of 1/10 Hz to 1/180 Hz and their motility changes were examined 24 hr later. On the drug experiment, prior to drug administration, animals received shocks (1/10 Hz) and their motility changes were examined 24 hr after the shock-delivery. The number of rats in each of the groups (shocked and non-shocked) of saline, diazepam, and apomorphine consists of 15, 5, and 7 rats, respectively. Apomorphine-HCl (Dainippon Pharmaceutical Co. Osaka, Japan) was dissolved in a solution containing 0.9% NaCl and 0.1% ascorbic acid. Diazepam (Yamanouchi Pharmaceutical Co. Tokyo, Japan) was suspended in a vehicle of 0.5% acacia. Animals were injected IP 15 min prior to the motility testing, in the following sequence: saline, apomorphine-HCl 0.1, 0.5, 1 mg/kg, diazepam 1, 2.5, 5 mg/kg.

On the study of the effect of environmental condition on motility, animals received shocks (1/10 Hz) as described above but their motility was measured by the Animex (Farad Electronics, Sweden) 24 hr after the shock-delivery.

RESULTS

Effect of Shock Frequency on Suppression in Motility of Shocked Rats

Figure 1 shows a relationship between the frequency of shocks and suppression in motility of rats at 24 hr after shocks. Under the 1/180 Hz frequency, rats received shocks at 3rd and 6th min after being placed in the cage during a 9-min period. Rats received 5 shocks at 1/90 Hz, 17 shocks at 1/30 Hz, and 53 shocks at 1/10 Hz frequencies. All rats that received shocks exhibited a marked suppression in motility when they were placed in the cage in which animals received electric shocks, and their total motility was reduced to 4.1–8.1% of that of the non-shocked rats. The motility of the non-shocked rats on day 2 was about 65% of day 1. The motility of shocked rats (under 1/10 Hz) during the third 3 min-period tended to increase as compared with that during the first and the second 3-min periods (Fig. 2). A Similar tendency was also seen in the other frequency ranges of shock. On the contrary, the non-shocked rats, exhibited a greater motility rate in the first 3 min-period and gradually decreased in the other periods. The shocked rats exhibited following behavior: immediate movement, quick movements of their heads from side to side, fixed movements of their four legs as soon as they find their positions, and decreases in spontaneous motility.

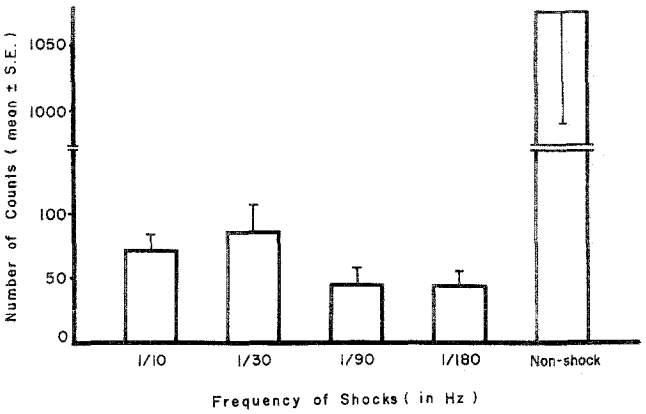

FIG. 1. Relationship between frequency of shocks and the degree of suppression in motility of shocked rats. Rats received shocks in test cage and 24 hr after the shock-delivery placed in the same cage in which rats received shocks to examine motility changes. The number of counts shows the motility during 9 consecutive min.

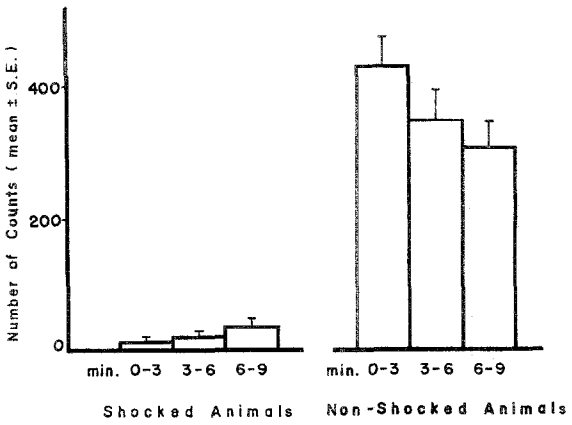

FIG. 2. Changes in motor activity of shocked rats (1/10 Hz) and non-shocked rats. Rats received shocks in test cage and 24 hr after the shock-delivery placed in the same cage in which rats received shocks. Motor activity was measured each 3 min-period during 9 min.

Effect of the Environment on Motility of Shocked Rats

The experiment was carried out to demonstrate whether the behavioral suppression, a marked reduction of motor activity when animal was placed in the same cage in which animal experienced shocks, is a conditioned response to the environment. Rats received brief shocks in the cage as described above and then the motility of rats at 24 hr after the shock-delivery were measured by Animex system, different situation from the cage in which animals received shocks. As shown in Table 1, shock experienced rats exhibited a similar degree of locomotor activity to that seen with the non-shocked rats. In addition, there was no difference between the behavior of shocked rats and that of the non-shocked rats in their home cage before and after the testing. The behavioral suppression is a kind of conditioned suppression to the environment previously associated with shock-delivery.

Effect of Diazepam on the Conditioned Suppression

As shown in Fig. 3, even a higher dose of diazepam (5 mg/kg), neither attenuated the conditioned suppression in motility of shocked rats, nor produced marked sedation and muscle relaxation. Motility of non-shocked rats was reduced, during entire testing period, by about 50%, with this dose of diazepam. Diazepam at the range of 1–5 mg/kg did not reverse the reduction of the conditioned suppression of motility in the first 3 min-period. The motility rate in the first

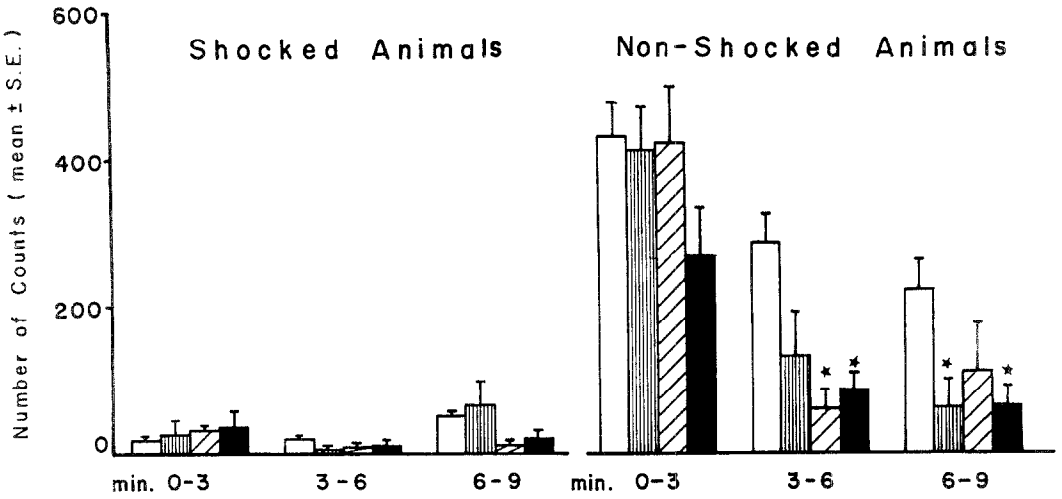

FIG. 3. Effect of diazepam on motor activity of shocked rats and non-shocked rats. Saline-pretreated group: (□), diazepam-pretreated group: (▥); 1 mg/kg, (▨); 2.5 mg/kg, (■); 5 mg/kg. Levels of significance: $*p<0.05$ as compared with the respective saline-pretreated group (Student's t-test).

TABLE 1

EFFECT OF THE ENVIRONMENTAL CONDITION ON MOTILITY OF SHOCKED RATS

	No. of rats	No. of counts (mean ± S.E.)
Shocked animals	8	203.8 ± 33.9
Non-shocked animals	7	245.9 ± 46.1

The number of counts shows the motility in ANIMEX motility meter during 9 consecutive min.

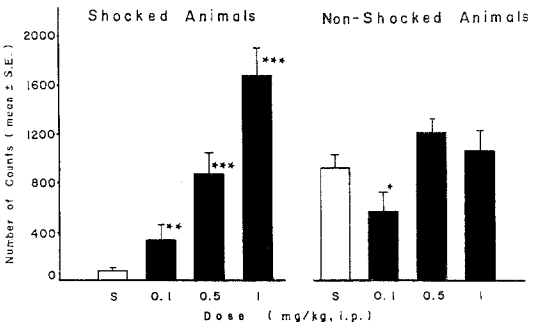

FIG. 4. Effect of apomorphine-HCl on motor activity of shocked rats and non-shocked rats. S=saline-treated group. The number of counts shows the motility during 9 consecutive min. Levels of significance: $*p<0.05$, $**p<0.01$, $***p<0.001$ as compared with the respective saline-pretreated group (Student's t-test).

3 min-period under diazepam, at doses of 1, 2.5 and 5 mg/kg, is almost the same as that of saline-pretreated group.

Effect of Apomorphine on the Conditioned Suppression

Although, apomorphine attenuated significantly the conditioned suppression in motility of shocked rats in a dose-related manner, a dose of 0.1 mg/kg, but no other, significantly decreased motility of the non-shocked rats ($p<0.05$) (Fig. 4). The reduction of the conditioned suppression in motility of shocked rats by means of apomorphine at 3 doses (0.1, 0.5, and 1 mg/kg) was significant, as compared with control ($p<0.01$, 0.001, and 0.001). Apomorphine (0.5 and 1, but not 0.1 mg/kg) enhanced gnawing behavior during the testing in the both groups of rats.

DISCUSSION

The present results indicate that shock experienced rats show a marked reduction in motility when placed in the environment in which the animals previously received shocks. The experiment of the effect of environmental condition on motility carried out the motility measurement by means of Animex system to exclude the conditioning of the grid floor and the acrylic rectangular cage. The shock experienced rats do not show any reduction of motility in the environment in which animals did not receive shocks. Furthermore, an anti-anxietic agent such as diazepam is known to induce a decrease in motor activity by both the infrared [27] and the magnetic test [19]. Thus, it is possible that the behavioral suppresson of shocked animals is a kind of conditioned response to the environment. It was found that the shock experienced rats froze immediately after being placed in the same test cage without the delivery of shocks, shook their heads slowly several times to the left and right, and then moved their legs slowly and carefully. These responses occurred in all shocked rats. When the degree of the behavioral suppression was investigated in relation to the shock frequency, the frequencies of 1/10 Hz to 1/180 Hz equally

produced a marked suppression of motility. In fact, the amount of suppression caused by 2 shocks and 53 shocks was not significantly different from each other. It is possible that the delivery of only 2 shocks at 1/180 Hz does not produce a consistent conditioned suppression, since the leakage of grids frequently occurred with excreta of animal during shock. Therefore, the results were obtained by using the frequency of 1/10 Hz. In the present experiment, the behavioral suppression lasted 28 days after the shocks delivered [27].

Diazepam (1, 2.5, 5 mg/kg) useful in clinical therapies of anxiety, did not affect the conditioned suppression, in spite of the doses of diazepam used which caused not only a marked reduction of the conditioned emotional response [1], but also a marked disinhibition of the punished suppression in rats [9] in the literatures. Motility of the non-shocked rats pretreated with the same dose of diazepam did not change in the first 3 min-period, though these dose ranges of diazepam reduced the motility of the non-shocked animals in the 2nd and 3rd 3 min-periods. A dosage of 5 mg/kg of diazepam caused slight sedation in rats 15 min after treatment in their home cage. Therefore, diazepam may not be involved in the reduction of the conditioned suppression in motility of shocked rats.

The conditioned suppression in motility may have occurred by the decrease of dopaminergic mechanisms, since the behavioral suppression is similar to catalepsy. In addition, apomorphine caused a marked reduction of the conditioned suppression in a dose related manner. On the contrary, apomorphine did not increase motility of the non-shocked animals in the present experiment.

The decrease of motility induced by apomorphine in the non-shocked rats may be related to the decrease of dopamine release from the terminal due to stimulation of the autoreceptor by the agonist [26]. Thus, the decrease of motility observed when the shock experienced rats were placed in the same environment may be related to the decrease of dopaminergic neuronal function. In other words, the decrease of dopamine release from the terminal due to stimulation of the autoreceptors by endogenous dopamine may be caused in shock experienced rats.

We reported that opiates, tricyclic antidepressants and MAO-inhibitor attenuate the conditioned suppression [29,30]. Haloperidol and chlorpromazine, having no effect on the disinhibition of punished suppression [10], are not effective in reducing the conditioned suppression [28]. Atropine (5 and 10 mg/kg), higher doses than applicable for cardiac conditioned suppression [15,20], and para-chlorophenylalanine induced a slight decrease in the conditioned suppression [27].

These results, together with the present findings, suggest that the conditioned suppression in shocked rats observed in these experiments does not correspond to the conditioned emotional response as described by Estes and Skinner [6] and is not related directly to anxiety, that diazepam is effective in a conditioned emotional response, and that dopaminergic mechanisms may be involved in the reduction of conditioned suppression.

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