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Effect of d-Amphetamine, Ethanol and Genever on Learning in the Rat[1]

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MERLO, A. B., H. E. M. FABIAN, E. CHEMERINSKI AND M. BILLIET. *Effect of d-amphetamine, ethanol and genever on learning in the rat.* PHARMAC. BIOCHEM. BEHAV. 4(3) 239–242, 1976. – The effect of oral administration of d-amphetamine (5 mg/kg), ethanol (2.5 g/kg), genever (equivalent to 2.5 g/kg ethanol) and the combinations d-amphetamine-ethanol and d-amphetamine-genever was studied on learning in the rat, using a shuttle-box in 4 sessions at 24 hr intervals. Acquisition was significantly favored by all treatments, being more constant in the 4 sessions when combinations d-amphetamine-ethanol and d-amphetamine-genever were administered.

d-Amphetamine Ethanol Genever Learning

IN our previous works [5,7] and in agreement with some authors [1,9] it could be seen that ethanol and beverages that contain ethanol are capable, in certain experimental conditions, to favor acquisition in the rat. So that acquisition in a shuttle-box was favored in rats given ad lib ethanol or genever as the only source of beverage (for 3 days prior to the first learning session and throughout the test period) [5,7]. In the present paper, the effect of d-amphetamine, ethanol, genever and the combinations of d-amphetamine-alcoholic beverages (ethanol or genever) was studied on rat acquisition in a shuttle-box.

METHOD

Conditioning

Adult male (Wistar derived) rats, aged approximately 3 months and weighing 150–180 g, were used. They were kept in individual boxes at 24°C with a 12 hr light/dark cycle. The training box was divided into 2 compartments connected by a small opening allowing the passage of the rat from one compartment to the other. Each half of the box could be lighted independently by a 15 W lamp. Three habituation sessions were held every 24 hr during which, the rats were submitted to handling for 2 min; immediately after, each rat was placed in the shuttle-box and 5 electric shocks (2.5 mA; 50 Hz; 5 sec) were applied to the feet at intervals of 0.5–1.0 min.

Three days after the last habituation session, learning tasks were carried out daily between 10 a.m. and 1 p.m. for 4 consecutive days. Every day, 6 to 12 rats, including treated and controls, were conditioned. Each rat was tested at the same time each day.

The training consisted of placing the rat in one compartment of the box in the darkness which was then lighted. If the rat did not pass into the dark compartment within 5 sec after the light was switched on, it received an electric shock of the same intensity and frequency as in the habituation sessions until it moved into the dark compartment. The passage of the rat from the lighted compartment to the dark one within 5 sec of its being switched on, was considered a conditioned response. In each daily session, 50 light stimuli were given at intervals varying from 0.5–1.0 min. Every one of the 50 tests started by each light stimulus, constituted a trial.

The rats had free access to the balanced food (Forramez) and water till 90 min before and immediately after the four conditioning sessions.

Although the different treatments were administered by gastric tube the animals were not fasted, so as not to diminish the rate of normal metabolism of ethanol, as it was proved by Owens and Marshall [8].

Ninety and 60 min respectively before each conditioning session, different groups of rats received one of the following 6 treatments: (1) Water (5 ml/kg) + Water (8.4 ml/kg) (n = 11) (Control) (2) d-Amphetamine (5 mg/kg) + Water (8.4 ml/kg) (n = 12) (3) Water (5 ml/kg) + Ethanol (2.5 g/kg) (n = 10) (4) d-Amphetamine (5 mg/kg) + Ethanol (2.5 g/kg) (n = 12) (5) Water (5 ml/kg) + Genever (equivalent to

[1] Genever is the Dutch equivalent of the English Gin. We use this term because the destillation processes involved in their preparation is different.

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2.5 g/kg ethanol) (n = 8) (6) d-Amphetamine (5 mg/kg) + Genever (equivalent to 2.5 g/kg ethanol) (n = 8). d-Amphetamine solution was given in a volume of 5 ml/kg and ethanol and genever in 8.4 ml/kg (both containing 30% w/v ethanol). Commercial genever (39% v/v ethanol) was diluted to 30% w/v ethanol. This alcoholic concentration was chosen considering that other authors [4,12] administered the same range of doses of ethanol to rats by the same via in concentrations of 20% w/v and 30% w/v in tap water. Tap water was used for control administration and for vehicle for solutions.

RESULTS

Conditioning

The number of conditioned responses ($\bar{X} \pm SE$) of each group per block of 10 trials is represented in Fig. 1, with *t* test for statistical significance.

The results of treated groups were compared with the control group.

The amphetamine treated group performed a significantly increased number of conditioned responses throughout the first session. This effect could also be seen in the

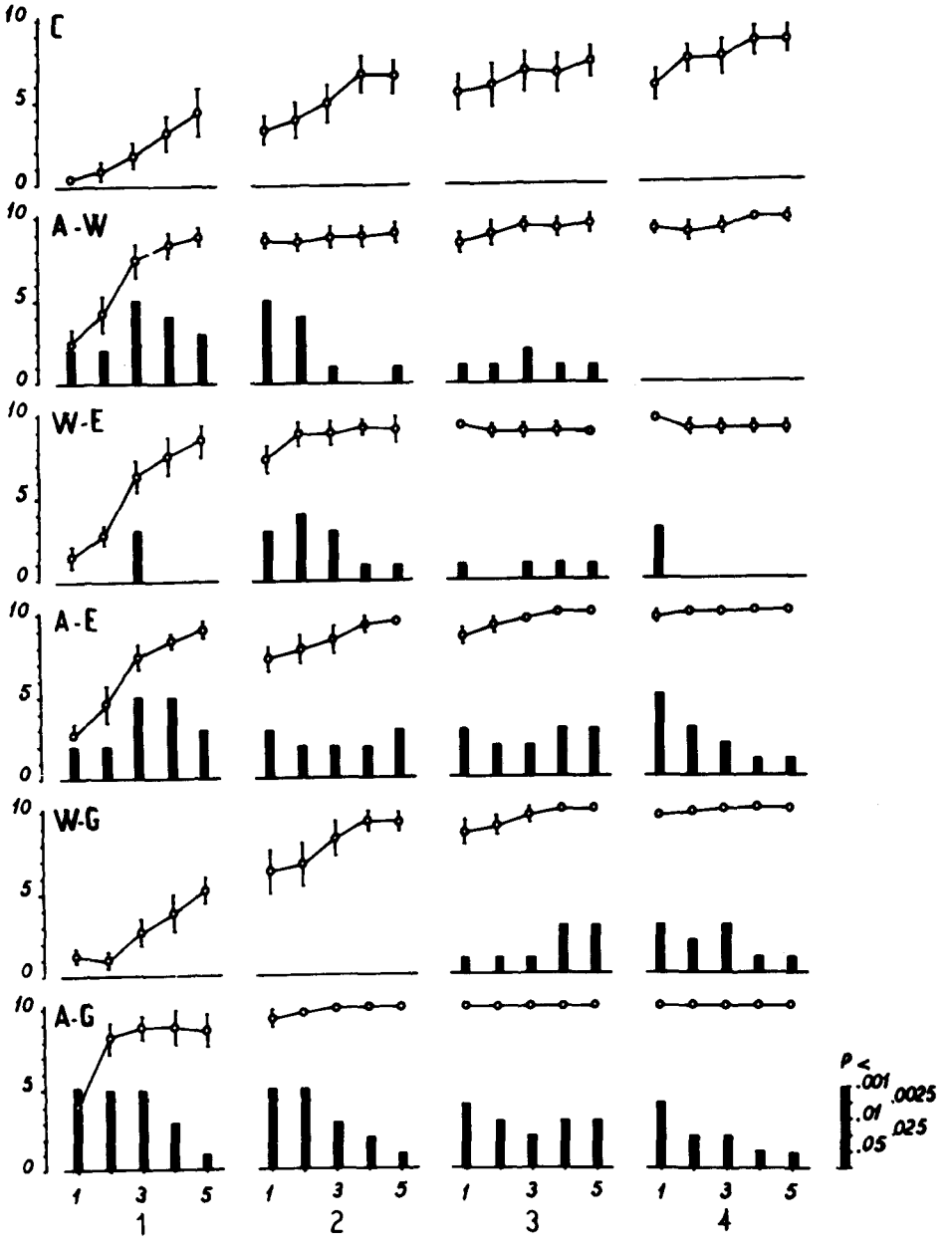

FIG. 1. Performance of the different groups of rats. C: control (water-water); A-W: d-amphetamine-water; W-E: water-ethanol; A-E: d-amphetamine-ethanol; W-G: water-genever; A-G: d-amphetamine-genever. Ordinates: number of conditioned responses ($\bar{X} \pm SE$). Abscissae: top: blocks of 10 conditioned stimuli; bottom: sessions. Black bars: level of significances in relation to control.

second session; it was maintained (but less significantly) in the third session and in the fourth session, the last one, it was not observed.

Acquisition of the conditioned response was significantly improved by ethanol in the third tenth of trials of the first session, during the second and third sessions and at the beginning, first tenth of trials, of the fourth sessions.

Though amphetamine and ethanol by themselves were capable of producing a significant increase in the number of conditioned responses, the combination amphetamine-ethanol produced a more constant and a more significant effect than each one per se. The most conspicuous effect was observed in the fourth session, where the combination was significantly effective throughout the whole session, while amphetamine alone did not produce a significant effect; neither did ethanol, except in the first tenth of trials.

Genever did not produce a significant effect on acquisition in the first 2 sessions, but favored it significantly in the third and fourth sessions.

The effect of amphetamine-genever was observed throughout the 4 sessions, similarly as it occurred with amphetamine-ethanol.

The average latencies (± SE) to perform the conditioned response for each group in each session is expressed in Table 1. There were no significant differences between control and treated groups in the corresponding session. In all the groups the average latency was significantly lowered (related to the first session) while the number of sessions increased.

TABLE 1

AVERAGE LATENCIES (± S.E.) OF DIFFERENT GROUPS OF RATS TO PERFORM THE CONDITIONED RESPONSE IN EACH SESSION

Groups	Latencies (sec) Sessions First	Second	Third	Fourth
Control: water-water	1.5±0.1	1.1±0.0	1.1±0.0	1.1±0.0
d-amphetamine-water	1.4±0.1	1.2±0.1	1.1±0.0	1.1±0.0
water-ethanol	1.3±0.1	1.2±0.1	1.0±0.0	1.0±0.0
d-amphetamine-ethanol	1.5±0.1	1.1±0.1	1.1±0.0	1.1±0.0
water-genever	1.3±0.1	1.1±0.0	1.1±0.1	1.0±0.0
d-amphetamine-genever	1.3±0.1	1.1±0.0	1.0±0.0	1.0±0.0

→Significant difference ($p<0.01$) related to the first session of the same group.

DISCUSSION

Acquisition in our rats was significantly enhanced by d-amphetamine. Learning facilitation by amphetamine was observed in animals [3] and humans [13]. In our learning test in the rat, the facilitating effect of d-amphetamine was observed in the first 3 sessions, but not in the last one (fourth session), where performance level was similar to control, suggesting a temporary improvement. This facilitation was most conspicuous in the first session when the conditioned response was beginning to be acquired; it lowered in the following 2 sessions when the high performance level in treated and control rats tended to reach a ceiling.

Psychomotor excitation and/or fatigue reduction might be the cause of the improved performance level, as suggested by other authors [13].

Ethanol favored the performance in the middle of the first session. This facilitation effect was more evident and continued during the second session; it was observed during the third and the beginning of the last one.

Genever favored acquisition significantly only in the last 2 sessions. Composition of nondiluted commercial genever (ml per cent v/v) determined by gas chromatography [7] was: methanol: 0.019; acetaldehyde: 0.002; ethanol: 38.560; n-propanol: 0.045; iso-butanol: 0.083; iso-amyl alcohol: 0.391. The different effect between genever and ethanol could be due to the volatile congeners of genever. We recently observed, using the same test, that the components of genever (by mixing the volatile components in tap water, in the same proportion of diluted commercial genever) significantly improved the performance in the third and fourth sessions. The same solution, without ethanol, significantly impaired the performance in the first and second sessions; in the third and fourth sessions there were no significant differences with the control group (tap water).

In spite of the differences existing between the effects of ethanol and genever, the combination amphetamine-genever exerted a similar influence as amphetamine-ethanol. Latencies of the different groups to perform the conditioned response were similar and tended to decrease while the number of sessions increased.

The combination amphetamine-depressor drug was also used by other authors in experiments on behavior, where they observed a higher effect than when using each one alone. Thus, Weiss and Laties [14] evidenced it on a learning test in dog using the association amphetamine-pentobarbital. Combination ampehtamine-amobarbital also favored motility in the rat [11] and in the mouse [2], more than each one alone. This effect was ascribed to the fact that amphetamine increases motor activity at the same time that barbiturates lower fear [10,11]. Though ethanol could also decrease fear, in our experiment it was probable that the facilitating effect of ethanol was not due to fear lowering as much as to the following: ethanol and genever alone had little effect on the performance of conditioned responses in the first session, when fear to punishment (shock) would be maximal according to Kimble [6], because avoidance response is beginning to be acquired. The effect of combination amphetamine-ethanol is more significant in the last session where fear would have been lowered, as the rat has already learned how to avoid the shock.

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