*Br. J. Pharmac.* (1969), 37, 79–86.

# Effects of chronic administration of (+)-amphetamine on maze performance of the rat

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1. The influence of (+)-amphetamine, given 1 min after each training session, on the performance of 124 rats in a Lashley III maze was measured every 48 hr.

2. The first three injections of the drug significantly improved the learning ability of naive rats.

3. With prolonged treatment, (+)-amphetamine strongly impaired the maze performance of these rats.

4. The chronic administration of (+)-amphetamine to previously trained rats produced the same adverse effect.

5. Amylobarbitone sodium given to previously trained rats 30 min before the training sessions completely blocked the adverse effect of (+)-amphetamine.

6. (+)-Amphetamine did not produce impairment of performance when given chronically 30 min before training sessions, to previously trained rats.

There has been increasing interest in the effects of central nervous system stimulants, such as strychnine (McGaugh & Thomson, 1962; Petrinovich, Bradford & McGaugh, 1965; Hudspeth, 1964; Ross, 1964; Franchina & Moore, 1968), picrotoxin (Breen & McGaugh, 1961) and pentylenetetrazol (Irwin & Benuazizi, 1966) on the phenomena related to learning and memory. In these studies the drugs were injected after the training trials, the time interval elapsing from trials to injections varying from seconds to one hour.

To our knowledge, up to the time when this work was started (1966), only one paper had been published (Kosman, 1964) showing the effect of amphetamine given post-trial. A second paper on the subject appeared in 1966 (Doty & Doty, 1966).

## Methods

The subjects were 124 male Wistar rats, 3 months old at the beginning of the experiments, which were housed in groups of four in wooden cages throughout the experiments. The basic apparatus consisted of a 4 unit Lashley III maze, similar to that used by Carlini & Kramer (1965). The pretraining apparatus consisted of a 160 cm long straight runway.

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During the experiments the animals were deprived of food for 24 hr alternating with 24 hr of free access to food. At the end of each period of deprivation they were given one training session. In a preliminary phase without drug administration and using the pretraining apparatus, the animals were trained until they reached the goal box in 2 sec or less. Six to eight training sessions were enough for all rats to reach this performance. Thus the preliminary phase took from 12 to 16 days.

The procedure with the Lashley III maze was as follows: after reaching the goal box, the rats were allowed 1 min of food reward, and were then removed to a small wooden cage where they remained for 1 min. The rats were then injected intraperitoneally with either 2·0 ml./kg of 0·9% sodium chloride solution (saline) or 1·0, 2·0 or 5·0 mg/kg of (+)-amphetamine, in a volume corresponding to that of the control solution. The running times and the number of errors were recorded. The maximum time allowed for an animal to negotiate the maze was 5 min. If a rat failed to reach the goal box in two consecutive sessions, it was considered to have failed altogether and was excluded from the rest of the experiment. Beginning 30 min after injections, the animals had free access to food for the next 24 hr. In summary, the animals were trained every 48 hr and received the drug 1 min after each training session.

Four series of experiments were performed and details of each series are given.

## Results

### *First series of experiments*

Amphetamine given post-trial had two opposite effects on the maze performance of naive rats (Table 1). Thus from the second to the fifth experimental sessions, amphetamine-treated rats performed better than controls; from the seventh session onwards, however, the treatment began to affect the performance adversely. At the last (twelfth) experimental session the performance of control animals was $0·8 \pm 0·6$ for errors and $8·7 \pm 4·6$ sec for running times, whereas rats treated with amphetamine 1 and 5 mg/kg had, respectively, performances of $1·6 \pm 1·2$ and $4·0 \pm 3·2$ errors, and $27·7 \pm 29·1$ and $68·4 \pm 66·2$ sec running times. This deterioration in performance was particularly evident in three rats treated with 5·0 mg/kg which were not able to reach the goal box within 5 min in the last three experimental sessions, though they had reached it in the fourth, fifth and sixth training sessions.

TABLE 1. *First series of experiments: effects of (+)-amphetamine given post-trial on maze performance of naive rats*

| Treatment | No. of animals | To criterion* E | To criterion* T | No. of rats reaching criterion | No. of rats with decay of performance | No. of rats failing to reach goal box |
|---|---|---|---|---|---|---|
| 0·9% NaCl | 11 | 43·8±13·6 | 559·8±179·0 | 10 | 0 | 0 |
| (+)-amphetamine 1·0 mg/kg | 11 | 24·3±12·9† | 298·7±204·1† | 7 | 2 | 0 |
| (+)-amphetamine 5·0 mg/kg | 12 | 26·2±8·44† | 321·2±37·4† | 9 | 7 | 3 |

The animals were given twelve training sessions in the Lashley III maze. The criterion for learning was two consecutive sessions with at most one error in each.

* The first experimental session was not included because it was made before the first administration of drugs; E, Average (±S.D.) of total number of errors made in reaching the criterion of learning; T, average of total running-time (sec) in reaching the criterion (±S.D.) † Student's *t* test: significantly different from controls ($P<0·05$).

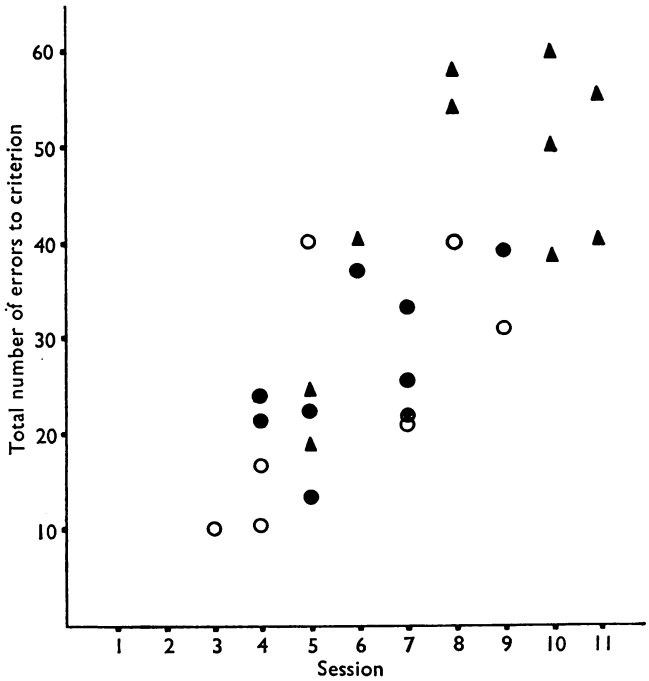


FIG. 1. Training sessions and errors made in reaching the criterion, by rats injected with 0·9% sodium chloride solution (▲), or (+)-amphetamine 1·0 mg/kg (○) and 5·0 mg/kg (●) 1 min *after* trials. Each symbol represents one animal. Note that most amphetamine-treated rats learned to negotiate the maze in fewer sessions and with fewer errors than controls.

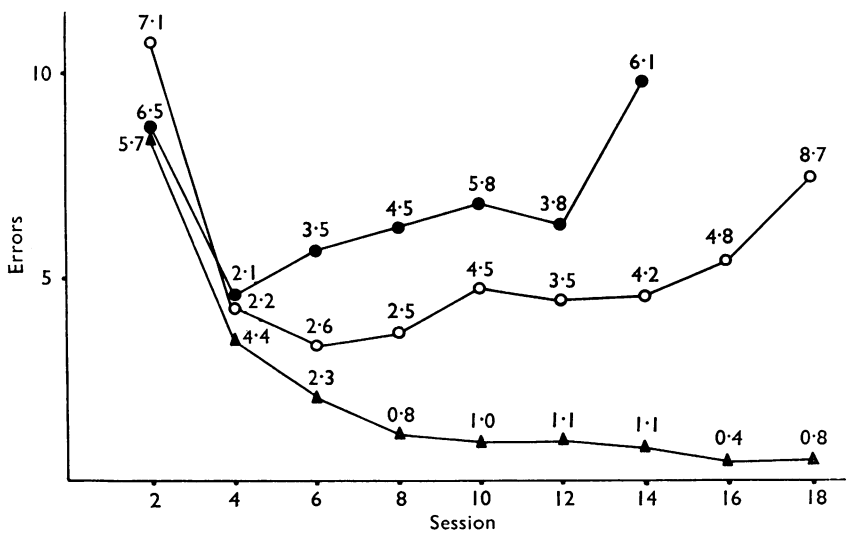


FIG. 2. Performance in a Lashley III maze of rats injected post-trial with control solution (▲), or (+)-amphetamine 1·0 mg/kg (○) and 5·0 mg/kg (●), from session 2 to 18. The animals had previously been given three training sessions without drug administration. Note that after the sixth session amphetamine treatment began to affect the performance adversely. Numbers above symbols represent the standard deviations.

TABLE 2. *Second series of experiments: effects of (+)-amphetamine given post-trial on maze performance of partially trained rats*

| Treatment | No. of animals | First phase Training sessions 2–5 E* | 2–5 T* | 6–18 E* | 6–18 T* | No. failing to reach goal box | Second phase Training sessions 1–9 E* | 1–9 T* | No. failing to reach goal box |
|---|---|---|---|---|---|---|---|---|---|
| Saline | 17 | 4·4±2·3 | 41·0±23·2 | 0·9±0·9 | 7·0±2·8 | 0 | 1·8±1·0 | 14·3±10·4 | 0 |
| (+)-amphetamine 1·0 mg/kg | 20 | 5·2±2·8 | 52·9±29·3 | 4·8±7·3† | 89·1±62·0† | 9 | 2·3±1·0 | 25·2±12·7† | 0 |
| (+)-amphetamine 5·0 mg/kg | 18 | 6·1±3·4 | 71·3±52·4† | 7·3±2·5† | 160·0±65·0† | 14 | 2·8±1·4† | 33·8±16·8† | 0 |

The rats were initially given three sessions in the Lashley III maze without drug administration. First phase: eighteen training sessions with drugs; second phase: nine training sessions without drugs after the animals had been allowed 2 months' rest, without sessions or injections.

* Average number of errors (±S.D.) (E) and running-times (±S.D.) (T) per session, from sessions 2 to 5, 6 to 18 (first phase) and 1 to 9 (second phase).

† Student's *t* test: significantly different from controls ($P<0·05$).

TABLE 3. *Third series of experiments: effects of amylobarbitone 20 mg/kg given 30 min before and (+)-amphetamine 2 mg/kg given 1 min after sessions on maze performance of well-trained rats*

| Training session | Treatment: Saline-saline (seven rats) E | Saline-saline (seven rats) T | Amylobarbitone-saline (eight rats) E | Amylobarbitone-saline (eight rats) E | Saline-amphetamine (ten rats) E | Saline-amphetamine (ten rats) T | Amylobarbitone-amphetamine (ten rats) E | Amylobarbitone-amphetamine (ten rats) T |
|---|---|---|---|---|---|---|---|---|
| Control 29 (no drug) | 0·0 | 5·1±1·0 | 0·0 | 5·5±0·9 | 0,0 | 4·9±0·7 | 0·0 | 5·2±1·7 |
| 1 | 0·0 | 5·1±1·2 | 1·3±1·7 | 14·2±17·8 | 0·0 | 5·2±1·1 | 0·3±0·4 | 14·8±23·4 |
| 2 | 0·0 | 5·1±1·2 | 0·5±0·6 | 11·0±9·7 | 0·7±1·4 | 8·6±5·7 | 0·6±1·1 | 8·1±5·4 |
| 4 | 0·0 | 5·5±3·5 | 0·0 | 5·6±1·9 | 1·6±2·7 | 19·0±19·3 | 0·9±1·0 | 8·8±5·4 |
| 6 | 0·0 | 5·2±1·7 | 0·0 | 4·0±0·3 | 1·8±2·1 | 21·0±29·4 | 0·1±0·3 | 5·5±1·8 |
| 8 | 0·0 | 5·4±1·1 | 0·2±0·4 | 4·6±1·5 | 1·8±2·2 | 21·6±34·3 | 0·2±0·6 | 7·1±6·9 |
| 10 | 0·0 | 5·8±2·6 | 0·1±0·2 | 5·2±1·8 | 2·9±3·1 | 62·0±81·4 | 0·7±1·5 | 15·3±28·1 |
| 11 | 0·3±0·45 | 5·5±1·5 | 0·0 | 4·5±0·8 | 2·2±2·6 | 73·3±77·5 | 0·0 | 4·6±1·8 |
| 12 | 0·0 | 4·4±1·3 | 0·0 | 4·0±1·0 | 1·9±2·4 | 54·3±67·9 | 0·0 | 5·0±1·7 |

The animals were previously submitted to twenty-nine pre-training sessions before drug treatment. Treatment consisted of twelve sessions in which saline or amylobarbitone sodium 20 mg/kg were injected 30 min before training followed by a second injection of either saline or (+)-amphetamine 2 mg/kg 1 min after reward. The first row refers to the performance of rats at the last (twenty-ninth) session before drug administration. Number of rats in each group are indicated within parenthesis. The third, fifth, seventh and ninth training sessions have not been entered in the table because they were very similar in value to their preceding sessions. E, Average number of errors ±S.D.; T, Mean running time per session ±S.D.

The improvement of maze learning ability caused by amphetamine is shown in Fig. 1 and Table 1, where it can be seen that treated rats reached the learning criterion (two consecutive sessions with at most one error in each) with fewer sessions and errors than controls.

### *Second series of experiments*

Doty & Doty (1966) reported an improvement in learning by rats after post-trial administration of amphetamine. A second series of experiments was therefore carried out to analyse further the deleterious effect of amphetamine seen in the later stages of our first series of experiments.

Figure 2 shows that after the sixth experimental session with amphetamine administered post-trial, the performance of the rats began to deteriorate. This decay in performance became accentuated until nine out of twenty and fourteen out of the eighteen rats treated respectively with (+)-amphetamine 1·0 and 5·0 mg/kg failed to reach the goal box within 5 min (Table 2).

Table 2 (first phase) shows the average errors and running-times from sessions two to five and six to eighteen ; it can be seen that, from the sixth to the eighteenth session, the number of errors and the running times of treated rats were five to twenty times greater than that of controls. This difference would have been greater still if animals failing to reach the goal box had been kept in the experiment.

From the sixth training session onwards, it was possible to observe differences in the gross behaviour of the amphetamine-treated animals. Thus, on reaching the door of the goal box, they hesitated in front of it ; several times they returned to the alleys instead of entering the goal box. Once inside it, they usually did not eat the food, but stayed quiet or explored the box.

Table 2 (second phase) shows the performance of these rats after a rest period of 2 months. Then all animals were able to reach the goal box ; control and rats given 1·0 mg/kg amphetamine performed equally well when errors were taken as measures of their performance. However, rats treated with 5·0 mg/kg made a number of errors and their running times were significantly greater than those of controls.

### *Third series of experiments*

These experiments were undertaken to verify whether amylobarbitone could counteract the adverse effect of chronic post-trial administration of (+)-amphetamine. Thirty-five rats were previously given twenty-four sessions in the Lashley III maze to reach a criterion of no errors in two consecutive sessions ; after that five more sessions followed in which the animals received an injection of saline 30 min before training. Then the animals were randomly divided into four groups and drug administration was started. Treatment consisted of an injection of either amylobarbitone sodium 20 mg/kg or saline 30 min before training and a second injection of either (+)-amphetamine 2 mg/kg or saline 1 min after reward. Table 3 summarizes the results.

It can be seen that animals which received saline twice, 30 min before and 1 min after sessions (saline–saline ; second and third columns), performed equally well throughout the twelve sessions ; amylobarbitone given 30 min before trials produced an effect which disappeared after the fourth administration (amylobarbitone–saline ; fourth and fifth columns).

Rats which had received saline 30 min before followed by (+)-amphetamine 1 min after sessions, as expected, showed a progressive decay of performance beginning in the fourth session (saline–amphetamine; sixth and seventh columns); three of the animals failed to reach the goal box. Finally, the last two columns (amylobarbitone–amphetamine) show that amylobarbitone 20 mg/kg given 30 min before each session completely blocked the adverse effects of post-trial (+)-amphetamine.

*Fourth series of experiments*

These were performed in order to verify whether (+)-amphetamine injected before trials, would have the same adverse effect on performance. For this experiment, the seven saline–saline and the eight amylobarbitone–saline rats used in the third series were used, after a rest period of 15 days. Seven of these rats (three saline–saline and four amylobarbitone–saline) served as controls and received saline; the remaining eight animals were injected with (+)-amphetamine 2·0 mg/kg. In both groups injections were given 30 min before training sessions. Table 4 shows that after ten sessions amphetamine-treated animals performed as well as controls, and showed no signs of the adverse effect observed when the same drug was administered 1 min after the reward.

## Discussion

The results of the experiments described indicate that (+)-amphetamine injected pre- and post-trial has different effects on the performance of well-trained rats in a Lashley III maze. Thus, whereas pre-trial injections had no effect (Table 4), post-trial administration impaired the performance of the rats (sixth and seventh columns of Table 3). This impairment was also evident in partially trained animals (Table 2). On the other hand, the adverse effect of (+)-amphetamine was completely prevented by pre-trial administration of amylobarbitone (Table 3).

The adverse effect of post-trial (+)-amphetamine given chronically deserves more attention. Thus, when administered to naive rats, impairment was preceded by improvement in performance. This improvement, observed from the second to the fifth injections (Fig. 1 and Table 1) is in accordance with the observations of Kosman (1964) and Doty & Doty (1966) who found, respectively, that amphetamine improved the performance of mice in a water maze and that of rats in simple avoidance and discriminated avoidance problems. However, with prolonged post-trial administra-

TABLE 4. *Fourth series of experiments: effects of (+)-amphetamine given 30 min before trials on maze performance of well-trained rats*

| Training session | Saline (seven rats) | | Amphetamine (2 mg/kg) (eight rats) | |
|---|---|---|---|---|
| | E | T | E | T |
| 1 | 0·0 | 4·2±0·9 | 0·0 | 4·3±1·3 |
| 2 | 0·0 | 4·4±1·2 | 0·0 | 4·1±1·0 |
| 4 | 0·0 | 4·4±1·1 | 0·0 | 4·2±1·2 |
| 6 | 0·1 | 5·8±1·9 | 0·0 | 5·1±1·0 |
| 8 | 0·0 | 5·1±1·6 | 0·1 | 4·8±1·7 |
| 10 | 0·0 | 5·2±1·4 | 0·0 | 5·0±1·4 |

The third, fifth, seventh and ninth experimental sessions have not been entered in the table because they were very similar in value to their preceding sessions.
E, Average number of errors per session; T, mean running time per session ±S.D.

tion of (+)-amphetamine, the adverse effect on performance appeared (Fig. 2). Thus, in the first three series of experiments, after the fourth to the sixth administration, the animals given amphetamine showed a deterioration in their performance, hesitated in front of the goal box or returned to the alleys, instead of entering the goal box to such an extent that several animals did worse than they had done at the beginning of the experiments.

The anorexic effect of amphetamine could have contributed to the adverse effect, because hunger was the drive used ; if hunger had been reduced animals might not have performed so well. We do feel, however, that this was not the case. Food was given to the animals for 24 hr, beginning 30 min after the injections, then they were deprived of food for 24 hr. Therefore, if the animals had eaten less when they were supplied with food, their hunger would have been greater at the beginning of the next session. Thus treated animals would have had a stronger drive and should have performed better. It was also observed that the weight of amphetamine-treated animals increased compared with that of controls, throughout the experiments, which indicates that the drug did not reduce food intake. It has been reported that doses of 5 mg/kg or less of (±)-amphetamine caused only a transient loss of weight or even weight gain (Ehrich & Krumbhaar, 1937 ; Shapiro & Freedman, 1957). Finally, if the anorexic effect of the drug was responsible, it should also have manifested itself in the fourth series of experiments, when the same dose of amphetamine was given 30 min before sessions.

The adverse effect of chronic administration of (+)-amphetamine on maze performance might have been due to effects such as those obtained by Utena, Ezoe, Kato, & Hada (1959) and Takahashi & Akabane (1960) on brain enzyme concentrations. This hypothesis, however, does not agree with the results reported here. Thus, after withdrawal of the drug, the adverse effect wore off after 2 months (Table 2) even for the rats that had failed to reach the goal box. Furthermore, if this were the case, the same adverse effect on maze performance should also have been observed in the rats of the fourth series of experiments (Table 4), which received the drug before sessions.

On the other hand, several facts suggest that post-trial amphetamine may act as an aversive stimulus. For example, both the hesitation of the animals in front of the goal box and the longer running-times of the post-trial amphetamine-treated animals suggest this ; Barry & Miller (1962) measured the conditioned fear caused by a conflict between approaching food and avoiding aversive shocks by the increase in running times of rats in a telescope-alley. Finally, the mitigation of the adverse effects by amylobarbitone, which is able to reduce conditioned fear (Miller, 1964) also suggests that post-trial amphetamine may have acted as an aversive stimulus. Therefore, the presentation of an aversive stimulus (post-trial amphetamine) after a positive reinforcing stimulus (food), could have set up an approach-avoidance conflict. The absence of adverse effects when amphetamine was given before training trials is in accordance with this hypothesis.

The observed effect that pre-trial injections of amylobarbitone, mitigated the adverse effect produced by (+)-amphetamine given post-trial, is of interest in relation to the drug interaction studies carried out by Steinberg (1964) and Rushton & Steinberg (1964) with amphetamine-barbiturate mixtures.

This work was partially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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(*Received September* 27, 1968)