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An Analysis of Visual Object Reversal Learning in the Marmoset After Amphetamine and Haloperidol

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RIDLEY, R. M., T. A. J. HAYSTEAD AND H. F. BAKER. *An analysis of visual object reversal learning in the marmoset after amphetamine and haloperidol.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 345–351, 1981.—The effect of amphetamine and haloperidol pretreatment on visual object reversal learning was assessed in the marmoset. Amphetamine induced perseverative responding demonstrated by high reversal learning scores and worse than chance performance in the early stages of reversal. This perseverative responding was prevented by pretreatment with haloperidol. Haloperidol, either alone or in conjunction with amphetamine caused a mild, non-perseverative impairment on reversal learning only.

Amphetamine Haloperidol Primate Reversal Learning

OBJECT discrimination reversal learning may be used to assess an animal's ability to modify its behaviour in response to changing reward contingencies. Impairment on performance of this task may result from a disruption of perceptual analysis of the object stimuli, inadequate response control, or from a dysfunction of attentional or reinforcement mechanisms. The pattern of errors comprising the impairment may suggest where the fundamental inability lies.

The ability to perform object discrimination reversal learning has been demonstrated in monkeys including macaques [8] and marmosets [3]. It has been shown to be disrupted by lesions of the prefrontal cortex [6], particularly orbitofrontal cortex [13] in rhesus monkeys. These animals had difficulty in relinquishing an established choice in favour of a newly rewarded object. Monkeys with lesions of inferotemporal or foveal prestriate cortex were also impaired on visual discrimination reversal learning [11]. In this case impairment consisted of both perseverative responding and slower acquisition of the new association. In cats [23] and rats [1] lesions of frontal cortex or the hippocampal/fornix system have been found to result in a general impairment in the early stages of serial object reversal learning. While it is clear that reversal learning requires both frontal and posterior association cortex and limbic structures, the effects of further subcortical lesions or of drug administration have not been assessed on this particular task. In these experiments we consider the effects of manipulation of the dopaminergic system by amphetamine and haloperidol on visual reversal learning in the marmoset.

METHOD

Animals and Apparatus

Four laboratory born adult marmosets (*Callithrix jacchus*, 3 ♀, 1 ♂) weighing 250–350 g served as subjects. Animals were housed individually and fed bread and pellet chow after training each day to maintain a balanced diet. All animals had previously been subjected to extensive visual discrimination training using red and white stimulus lights in a modified rat operant apparatus [17, 19, 24] and choice preference testing [18] in a small Wisconsin General Test Apparatus [7] which was also used in these experiments. Throughout training animals were presented with trials on which a screen was raised to reveal 2 small plastic figures covering 2 small food wells. One food well contained a 3 mm cube of banana which the animal could obtain by displacing the appropriate object (Fig. 1). Touching the other object was not rewarded and was scored incorrect. The left/right position of the positive (rewarded) object was determined by a pseudorandom schedule [5]. Each trial lasted until the animal made a response. The intertrial interval during which the next trial was set up lasted about 15 sec. During reversal training 2 stimulus objects—'ballerina' and 'Indian' were used throughout. These objects had previously been used as the rewarded objects for discrimination performance testing in another experiment [18] where each animal had had 260–380 trials of 'ballerina' (rewarded) versus 'soldier' (unrewarded) and 'Indian' (rewarded) versus 'guard' (unrewarded).

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FIG. 1. Interior of Wisconsin General Test Apparatus showing marmoset retrieving the reward having displaced the stimulus. In this case the discrimination is 'guard' versus 'soldier'.

Reversal training, which lasted 12–15 days, continued until the animal could reliably learn one discrimination (task 1, e.g., with 'ballerina' rewarded) to a criterion of 5 consecutive correct responses followed immediately by learning its reversal (task 2, 'Indian' rewarded) to 5 consecutive correct responses in a total of <50 trials each day. Task 1 on each day consisted of task 2 of the previous day, i.e., the animal had to relearn the discrimination which it had performed last on the preceding day.

Drug Testing

Throughout drug testing animals were trained to perform task 1 and its reversal, task 2, to 5 consecutive correct responses, where task 1 was the same as task 2 of the preceding day. If an animal refused to complete task 2 it was trained to 5 consecutive correct responses on that task alone without drugs on the subsequent day. Drug testing restarted on the following day with relearning of that task. Drugs were administered by IM injections into the thigh in volumes of 0.1 to 0.2 ml. d-Amphetamine sulphate (Sigma) was dissolved in 0.9% sodium chloride; haloperidol (Searle) was diluted from injection ampoules.

Experiment 1: Effect of Amphetamine on Reversal Learning

Amphetamine or saline was given each day 20–30 min prior to testing on the tasks and in the order shown in Table 1. Scores on the two days at each drug dose were summed in an attempt to obviate any effects of drug order or daily variation. At each dose, one day's training consisted of 'ballerina' (rewarded) versus 'Indian' followed by 'Indian' (rewarded) versus 'ballerina' and the other day's training consisted of these tasks in the opposite order.

Experiment 2: Effect of pretreatment with Haloperidol in Reversal Learning

Haloperidol (or saline) was administered 30–40 min prior to testing in the order: 0.0 (saline) 0.0, 0.01, 0.01, 0.02, 0.02, 0.02, 0.02, 0.01, 0.01, 0.0, 0.0 mg/kg. 0.6 mg/kg d-amphetamine or saline were given 20–30 min prior to testing on alternate days. Performance scores on the two days of the same drug combination were summed as before. Reversal training proceeded as previously.

RESULTS

Unless otherwise indicated statistical comparison on each task was made between each drug dose and the appropriate saline condition and between task 1 and task 2 at each drug dose using a matched pair t-test with 3 df. The results of statistical analysis and probabilities are given in the appropriate figure legend.

TABLE 1
EXAMPLE OF TASK ORDER FOR EXPERIMENT 1

Day	1	2	3	4	5	6
Dose mg/kg d-amphetamine	0.0	0.3	0.6	0.6	0.3	0.0
Task 1	B v I	I v B	B v I	I v B	B v I	I v B
Task 2 (Reversal of Task 1)	I v B	B v I	I v B	B v I	I v B	B v I

B=ballerina; I=Indian. The first named object in each pair is rewarded.

The Effect of Amphetamine on Relearning and Reversal

Figure 2A shows mean learning scores, i.e., trials up to (but not including) 5 consecutive correct responses while Fig. 2B shows the mean total number of errors for Experiment 1 on task 1 (open circles) and task 2 (reversal, filled circles). The slight increase in learning scores on task 1 after amphetamine does not reach significance ($p<0.1$, 0.3 mg/kg, $p<0.2$, 0.6 mg/kg amphetamine). In Experiment 2 learning scores after amphetamine alone are also slightly elevated compared to saline for task 1 but again this does not reach significance ($p<0.1$) see Fig. 4. Learning scores on task 2 (reversal) after amphetamine are grossly elevated in Experiment 1 (Fig. 2A) and Experiment 2 (Fig. 4A). Comparison of the total number of errors shows that amphetamine has no effect on task 1 but greatly increases the number of errors on reversal learning (see Fig. 2B, Fig. 4C). Thus amphetamine clearly disrupts reversal learning but does not impair relearning of a task which was performed to the required criterion on the preceding day. It is possible that this occurs because animals make perseverative errors at the beginning of reversal training. This can be seen by looking at the number of trials before two consecutive correct responses were made (see Fig. 3A). If an animal were to perform randomly, an average of 4 trials (i.e., 2 trials×2 days at each dose) would be made *before* two consecutive correct responses would occur. (This was determined by computer stimulation.) It can be seen that for task 2 under saline and for task 1 the animals' performance differed little from chance initially but that under amphetamine many trials were required on task 2 before two consecutive correct responses were made. The large standard error under 0.6 mg/kg d-amphetamine is due mainly to one animal which performed 69 trials before making two consecutive correct responses.

Effect of Pretreatment with Haloperidol on Relearning and Reversal

Figure 4 shows that pretreatment with either 0.01 or 0.02 mg/kg haloperidol diminishes the effect of amphetamine on learning scores in reversal (task 2) but has no effect on task 1. Haloperidol, either alone or in combination with amphetamine appears to retard learning in reversal (filled circles). Figure 5 (which shows the number of trials, (A,B) and errors (C,D) to two consecutive correct responses) demonstrates that haloperidol abolishes the perseverative effect of amphetamine. Although under 0.01 mg/kg haloperidol significantly more errors are made on reversal than on learning

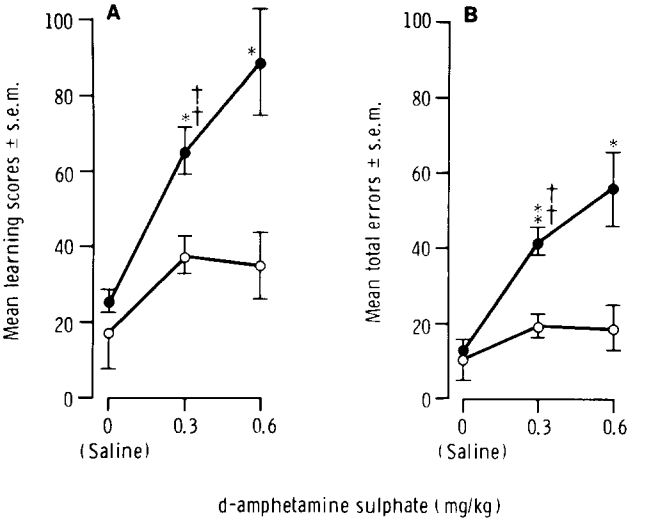

FIG. 2. Effect of amphetamine on (A) learning scores (mean trials up to but excluding 5 consecutive correct responses) and (B) mean errors on re-learning and reversal learning. ○=task 1 (re-learning). ●=task 2 (reversal of task 1). Ordinate: (A) mean learning scores or (B) mean errors ± SEM. Abscissa: dose of d-amphetamine sulphate given IM 20–30 min before testing. $*p<0.05$; $**p<0.01$ comparing scores under amphetamine with relevant saline control. $\dagger p<0.05$; $\dagger\dagger p<0.01$ comparing scores on task 1 with task 2; 2-tailed matched pair t-test with 3 df.

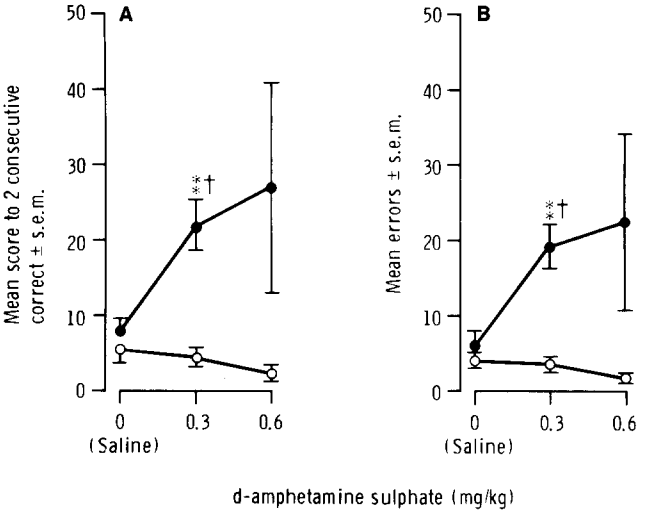

FIG. 3. Effect of amphetamine on (A) trials and (B) errors up to (but excluding) 2 consecutive correct responses. See Fig. 2.

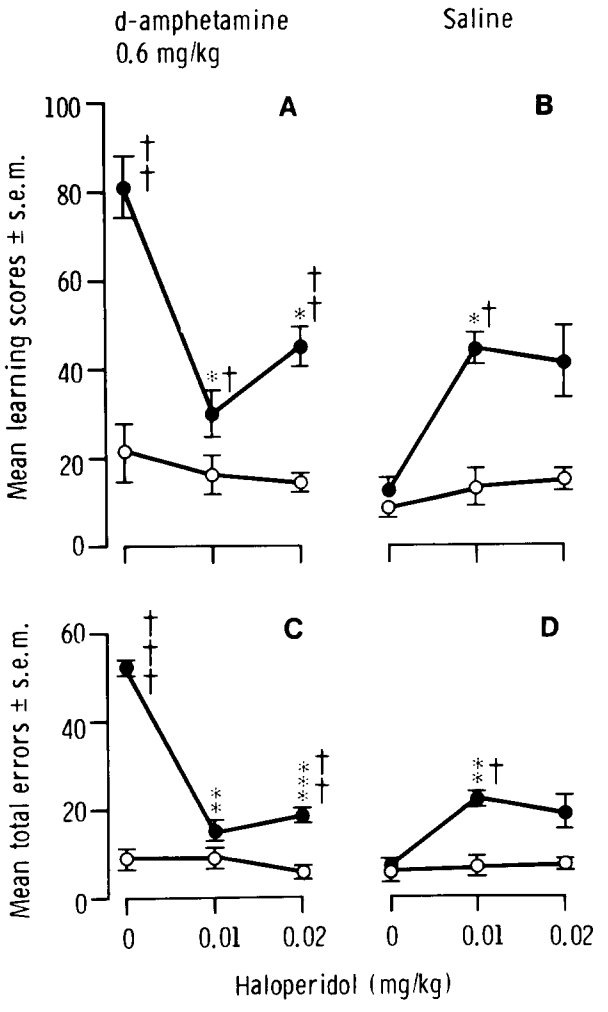

FIG. 4. Effect of haloperidol pretreatment on (A,B) learning scores (mean trials up to but excluding 5 consecutive correct responses) and (C,D) mean errors on re-learning and reversal learning. ○=task 1 (re-learning) ●=task 2 (reversal of task 1). Ordinate: (A,B) mean learning scores or (C,D) mean errors ± SEM. Abscissa: dose of haloperidol (mg/kg) given IM 30–40 min before testing. (0.6 mg/kg amphetamine (A,C) or saline (B,D) given 20–30 min before testing.) $*p<0.05$; $**p<0.01$; $***p<0.001$ comparing scores after pretreatment with haloperidol with the relevant amphetamine or saline control $\dagger p<0.05$; $\dagger\dagger p<0.01$; $\dagger\dagger\dagger p<0.001$ comparing scores on task 1 with task 2. 2-tailed matched pair t-test, 3 df.

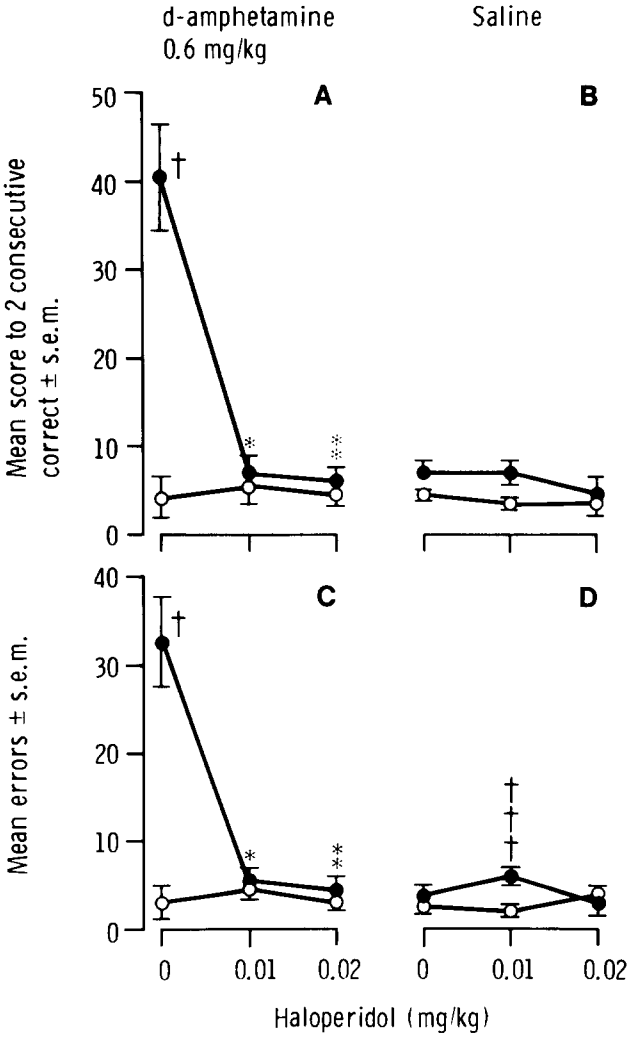

FIG. 5. Effect of haloperidol pretreatment on (A,B) trials and (C,D) errors up to (but excluding) 2 consecutive correct responses. See Fig. 4.

before 2 consecutive correct responses are made, the number of errors in reversal after haloperidol is not considerably greater than would be expected by chance. This effect of haloperidol cannot therefore be considered perseverative but may reflect the poor acquisition of reversal under haloperidol.

Learning Curves

Figures 6 and 7 show learning curves for each task for each drug condition. These were obtained by calculating the number of errors performed in blocks of 5 trials by all the animals. Thus the beginning of each curve represents the total performance of all four animals while the tail of each curve shows the errors performed by the slowest animal. Figure 6B illustrates the perseverative effect of amphetamine on task 2 since the number of errors performed remains above chance for about 20 trials after 0.3 mg/kg and 30 trials after 0.6 mg/kg amphetamine. The rate of learning after chance performance has been achieved is similar for saline and 0.3 mg/kg amphetamine. At the higher dose this rate of improvement is similar except for one animal whose sole performance is reflected in the errors after 50 trials. During task 1 (Fig. 6A) there is a suggestion of impaired re-learning after amphetamine illustrated by the performance between 5 and 15 trials, even though the final learning scores are not significantly different. The number of errors between trials

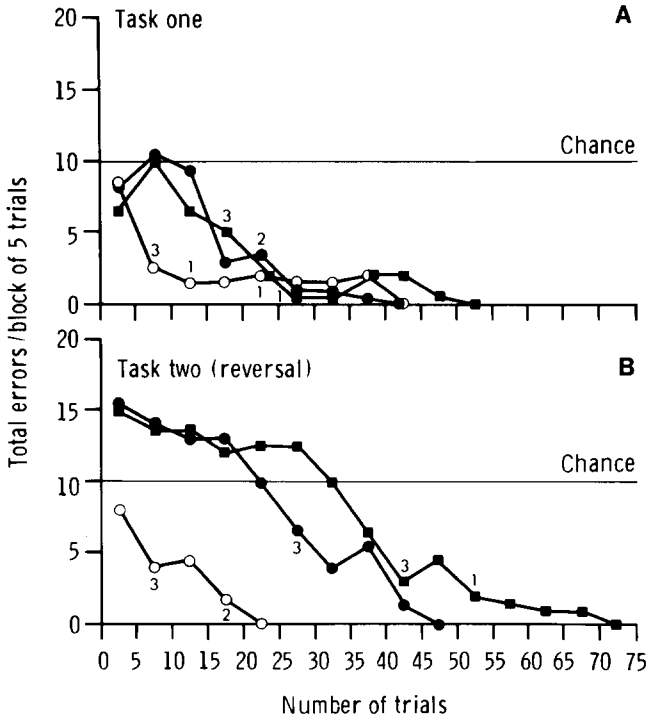

FIG. 6. Learning curves for (A) task one and (B) task two (reversal) for Experiment 1. The beginning of each curve shows the total errors in blocks of 5 trials made by all 4 animals (means of 2 days at each drug dose). Numbers in brackets indicate when fewer animals are involved as certain animals reach criterion and stop performing. ○=saline; ●=0.3 mg/kg; ■=0.6 mg/kg amphetamine.

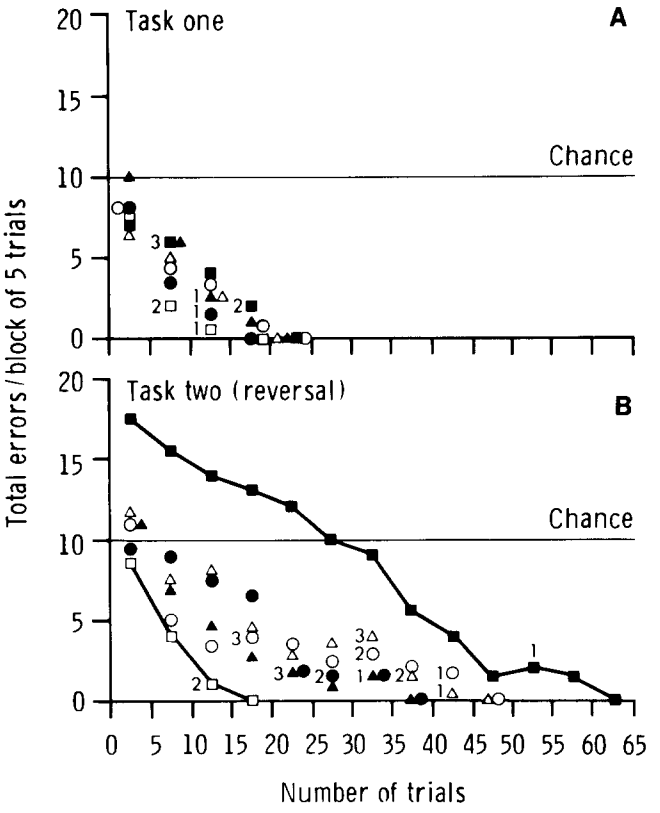

FIG. 7. Learning curves for (A) task one and (B) task two (reversal) for Experiment 2. □=saline/saline; △=0.01 mg/kg haloperidol/saline; ○=0.02 mg/kg haloperidol/saline; ▲=0.01 mg/kg haloperidol/0.6 mg/kg amphetamine; ●=0.02 mg/kg haloperidol/0.6 mg/kg amphetamine; ■=saline/0.6 mg/kg amphetamine. See Fig. 6.

5–10 after 0.3 mg/kg d-amphetamine is significantly greater than after saline ($p<0.05$). The difference over trials 10–15 cannot be estimated since 3 animals had by then completed training under saline. There is thus some suggestion of a re-learning impairment after amphetamine although this effect is not evident during Experiment 2 (Fig. 7A) when all animals had received more overall training. Figure 7B shows slower learning in reversal in the absence of a perseverative effect after haloperidol and the perseverative effect of amphetamine alone. It appears that amphetamine animals have to 'learn their way' back to chance performance as well as learning the appropriate stimulus associations. Figure 8 shows learning curves for the first 10 trials of each task where performance has been combined over each drug combination regardless of dose in Experiments 1 and 2. The perseverative effect of amphetamine is seen on reversal (B) since errors remain consistently above chance under amphetamine alone but drop to chance under all other conditions by the second or third trial. The lack of improvement in the early stages of re-learning task 1 can also be seen in Fig. 8A since by the end of the first 10 trials the animals show evidence of learning under all conditions except amphetamine alone.

DISCUSSION

That amphetamine may cause stereotypy or motor perseveration is beyond dispute [10]. In this experiment we have demonstrated that low doses of amphetamine may cause choice perseveration even where this is achieved through different response patterns (left/right choice). Since this effect may be blocked by pre-treatment with the relatively specific dopamine antagonist, haloperidol [14], it would appear to be mediated by dopamine. We are thus able to corroborate our previous finding [18], that dopamine is involved in higher order cognitive functions and that an appropriate dose of neuroleptic may alter these cognitive functions without disrupting the motor actions required to perform a response. It remains to be determined whether the cognitive perseveration found here is responsible for the motor stereotypy observed at higher doses.

Since amphetamine treated animals learn the first task of each day with only a trivial difficulty, their impairment on reversal cannot be ascribed to motor or simple perceptual loss. Thus they are either unable to overcome a response habit (a higher order response-organising impairment) or they fail to notice that the reward-associations of the stimuli

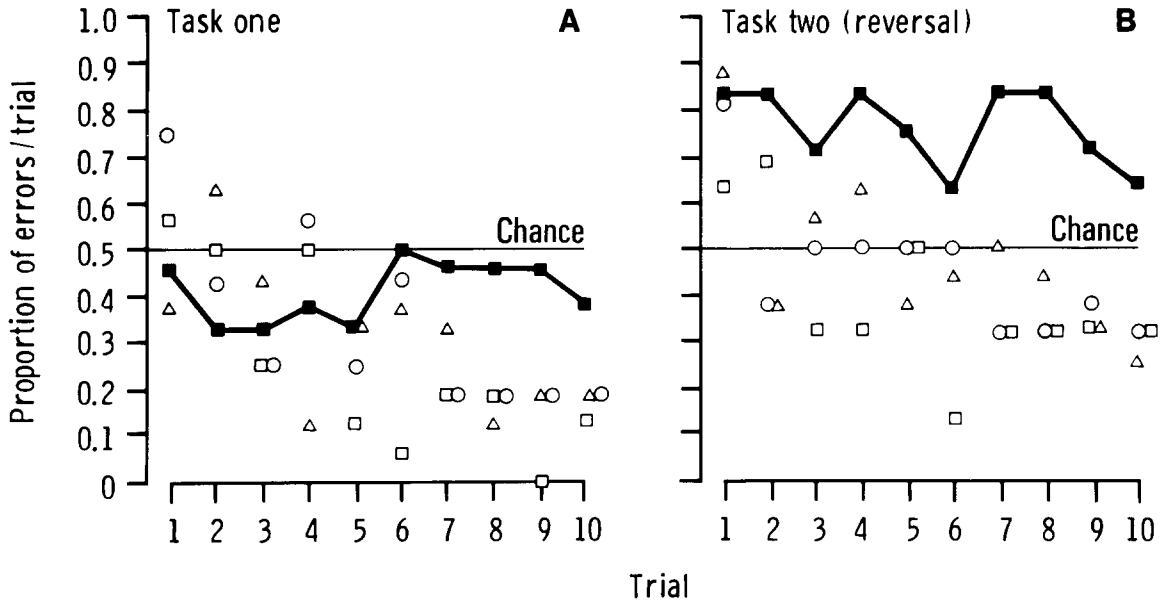

FIG. 8. Performance during the first 10 trials of (A) task one and (B) task two (reversal). Ordinate: proportion of errors/trial/drug combination averaged over Experiment 1 and 2, and each drug dose. ■=amphetamine alone; □=saline; △=haloperidol alone; ○=amphetamine and haloperidol.

have been reversed (a higher order perceptual/associative impairment). Behaviour in the first 10 trials of reversal (Fig. 8B) suggests that without amphetamine animals relinquish an association after only one or two non-rewarded responses before re-learning the new association. It is possible that two mechanisms are involved in reversal learning, one actively inhibiting a no longer appropriate response and the other acquiring the new association. If this were the case then perseveration after amphetamine could be due to a failure of the active inhibitory system alone. Such a loss of inhibition has also been used to describe the impairment of amphetamine treated marmosets on successive but not simultaneous visual discrimination [17]. Figure 7B suggests that amphetamine treated animals unlearn the inappropriate association at the same rate at which they then learn the new association; a result which might be expected in the absence of an active inhibitory system.

An involvement of dopamine in cognitive functions is suggested not only by recent studies of non-motor effects of stimulants such as amphetamine but also by certain effects of dopamine-depleting 6-OHDA lesions. Delayed spatial alternation, a complex task requiring cognitive integration of memory and spatial orientation, has been found to be disrupted by 6-OHDA induced depletion of dopamine in frontal cortex of monkeys [2], in striatum of rats [4] or by destruction of rising dopaminergic A10 neurones [22]. Furthermore, sensory neglect induced by unilateral 6-OHDA lesions of the substantia nigra [9,12] is considered to be neither a primary sensory nor motor deficit but rather to reflect a form of attentional failure [20]. That this sensory-motor disconnection may be compensated by appropriate conditioning [21] suggests that complex mechanisms of perceptual analysis and learning are involved.

At the highest level of cognitive processing the apparent dichotomy between stimulus-analysing and response-organising systems may be ill-conceived. Results of neurophysiological recording from unanaesthetized animals suggests that response-related and stimulus-sensitive units may be found in close proximity, the relative proportion of each depending on the cortical area under investigation [16]. In this case a loss of response-organising mechanisms and an apparent insensitivity or inattention to stimulus change may be equivalent in terms of mechanism.

In previous studies [17,24] we have pointed out the similarity between the effects of amphetamine administration and frontal or hippocampal lesions in monkeys. Although lesions of these areas resemble each other in many of their behavioural effects [23], a difference is apparent when considering the nature of the impairment on visual discrimination reversal learning. Pribram *et al.* [15] have shown that monkeys with amygdala-hippocampal lesions are impaired on reversal because of a long period of time spent performing at chance but that they both abandon their previous response habit and approach criterion rapidly. Gross [6], on the other hand, has shown that monkeys with frontal lesions perform most errors at the beginning of each reversal and only gradually return to chance performance. In this respect amphetamine treatment resembles the effect of frontal rather than hippocampal lesions.

If animals treated with amphetamine are unable to relinquish a choice of response when external conditions demand it, they may also be unable to switch from one choice of behaviour to another and thus become locked into a progressively more restricted behaviour pattern. Furthermore, a functional overactivity in dopamine systems in man may result in a comparable perseveration in cognitive processes.

Speculatively, this might result in an inability to relinquish a mode of thought or behaviour in the face of contrary external evidence, i.e., an obsessional or irrational thought disorder, and restricted behavioural repertoire.

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