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Validity of Head-Dipping as a Measure of Exploration in a Modified Hole-Board

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Abstract. To determine whether head-dipping could be validated as a measure of exploration a modified hole-board was developed with four holes in the floor, under which novel objects could be placed. Two criteria for considering head-dipping as a measure of exploration were proposed: firstly, that it should reflect novel aspects of the environment; secondly, that exposure to the hole-board should result in information storage. That head-dipping reflected novelty was indicated by the longer duration of head-dips on initial exposure if objects were present, and also on a second exposure when objects were introduced for the first time. Information storage was indicated by habituation on re-exposure to the hole-board. A significant positive correlation between head-dipping in the "four" and "sixteen" hole-boards was obtained for rats, but not for mice. This provided some indirect evidence that rat head-dipping in the "sixteen hole-board" also reflects exploration. (+)Amphetamine and alcohol were tested in the modified hole-board, and (+)amphetamine decreased and alcohol increased the frequency and duration of head-dips.

Key words: Validity – Hole-Board – Exploration – Mice – Rats (+)Amphetamine – Alcohol.

Introduction

The hole-board apparatus was first introduced by Boissier and Simon (1962) and since then has been extensively used to study drug effects (Bradley *et al.*, 1968; Dorr *et al.*, 1971; Nolan and Parkes, 1973; Valzelli, 1969, 1971; Wakely and O'Sullivan, 1969). Boissier (1965) distinguished curiosity and fear as two factors governing an animal's behaviour in a new situation, with escape reflecting the result of these two factors. Boissier and Simon (1964) acknowledge that repeated head-dips may reflect greater curiosity or a desire to escape, but the interpretation of single head-dips must also be questioned. They claim (Boisser *et al.*, 1964) that head-dipping does not reflect basal activity because the situation is new to the animal, but it is also necessary to distinguish between locomotor reactivity (Gross, 1968) and exploration – the latter being the behaviour by which an animal gains information about its environment (Halliday, 1968). Although the conceptual distinction between activity and exploration has been widely accepted (Archer, 1973; Hughes, 1972; Sheldon, 1968) this separation is not always easy to achieve in test situations using rodents.

Head-dipping may be a measure which reflects either or both of the factors activity and exploration, and it is the purpose of this experiment to determine whether empirical evidence can be obtained which validates it as a measure of exploration. The existing hole-board (Boissier and Simon, 1962) was unsuitable for this purpose for three reasons: firstly, there were too many holes for the animals to be able to discriminate between them; secondly, the density of holes was such that an animal could not display motor activity without coming into contact with a hole; and lastly, there was insufficient room to place objects under discrete holes. Thus a modified hole-board was developed, with only four holes in the floor, instead of sixteen. Objects could be placed underneath the holes, so that the features of the stimulus complex could be readily manipulated and the resultant effects on the animal's behaviour recorded.

Based on conclusions drawn from experiments by Blanchard *et al.* (1970), two criteria were used for establishing whether head-dipping reflects exploration. Firstly, head-dipping should reflect any novel aspects of the environment. In order to test this, objects were either introduced or removed on a second exposure to the hole-board, and the animals' responses to this change were noted. Secondly, exposure to the stimulus complex should result in information storage, which would be reflected in habituation on re-exposure to the hole-board.

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Experiment 2 investigated the correlation between head-dipping in the new apparatus and that in the "sixteen hole-board". If head-dipping is validated as exploration in the "four hole-board", a positive correlation would provide at least some indirect evidence that head-dipping in the "sixteen hole-board" also reflects exploration. To complete the validation of the new apparatus, in Experiment 3 the effects of two drugs, amphetamine and alcohol, were tested on head-dipping behaviour.

Experiment 1

Method

Subjects. Fifty-three male mice of the CFW strain, 15–20 g in weight, were used. They were divided into 5 groups – A, B, C and D containing 10 animals, and E containing 13. They were housed in groups of thirty before the start of the experiment and kept in a natural day-night cycle.

Fifty male hooded Lister rats, 250–400 g in weight, were used. They were divided into 5 groups – A, B, C, D and E, each containing 10 animals. They were housed in pairs and kept in a 12 hr light-12 hr dark cycle (lights on from 08.00–20.00 h).

All animals were kept in rooms maintained at a constant temperature of 21°C and were allowed *ad libitum* food and water.

Apparatus. The modified mouse hole-board was a wooden box with a floor 40 cm square and walls 27 cm high. There were four equally spaced holes in the floor, each 3 cm in diameter and 1.8 cm thick. The centre of each hole was 10 cm from the nearest wall.

The rat hole-board was also a wooden box with a floor 66 × 56 cm and walls 47 cm high. There were four equally spaced holes in the floor, each 3.8 cm in diameter and 1 cm deep. Two of the holes were 14 cm and the other two 17 cm from the nearest wall. The walls of both pieces of apparatus extended below the level of the floor, which was thus raised to a height of 12 cm.

In certain of the test conditions, objects were placed underneath the holes in the floor of the hole-board. These objects were: an aluminium pot containing matches; a brass weight; a rubber bung with a bar of soap on top; and a glass funnel with a tissue in the top. The top of each object was approximately 3 cm below the level of the hole. Pilot experiments had shown that these objects reliably elicited equal amounts of investigation.

Procedure. Each animal was placed singly in the centre of the board, facing away from the observer and its behaviour recorded for 10 min. A head-dip was scored if both eyes disappeared into the hole. The duration of time an animal spent looking down a hole was also measured using a stop-watch. Very brief, single head-dips were arbitrarily assigned a duration of 1 s, due to the inaccuracy of measuring such a short period with a stop-watch. Thus, for each animal, the total number and total duration of head-dips made was scored. Rearing and defaecation scores were also noted. After each trial, the floor of the apparatus was wiped and dried to remove traces of the previous path. Due to wide variations in motor activity and head-dipping throughout the day (File and Day, 1972), the mice were tested between 14.30 and 16.30 h and the rats were tested between 09.00 and 11.00 h and 16.30 and 18.30 h, half of each experimental group being tested at each time.

All 5 groups of animals were given two exposures to the hole-board separated by 24 hrs. For group A, there were objects under all the holes on both exposures (present-present condition). For group B, there were no objects under the holes on either exposure (absent-absent condition). For group C, there were objects under the holes on trial 1, but not on trial 2 (present-absent condition) and *vice versa* for group D (absent-present condition). For group E, there were no objects under any holes on trial 1, but there was an object (the glass funnel and tissue) under one hole on trial 2. The purpose of this last group was to act as a control for any non-specific rise in activity produced by the presence of objects.

Results

The means and standard errors of the total head-dip and duration scores were calculated for both exposure periods (these are shown in Table 1 and Fig. 1 respectively). Fig. 1 shows that both mice and rats spent significantly more time looking down the holes on trial 1 if there were objects placed underneath (for mice $t = 2.47$, $df = 38$, $P < 0.02$; for rats $t = 3.11$, $df = 38$, $P < 0.01$), even though they explored no more holes (see Table 1).

The difference between the mean scores on the two exposures were assessed by related t-tests (the t-values and probability levels are in Table 2). If the condition did not change from trial 1 to 2 the animals showed significant habituation on both measures, and significant habituation also occurred with the present-absent condition. In the absent-present condition, where objects were introduced for the first time on trial 2, the total head-dips score did not show habituation and the duration of head-dipping increased, indicating that the animals were spending more time looking down the holes at the objects.

For both mice and rats higher rearing scores were obtained on trial 1 if there were no objects (71.3 and 23.9 respectively) than if there were objects present (56.3 and 18.3 respectively), a difference which was significant for the mice ($t = 2.14$, $df = 18$, $P < 0.05$), but just failed to reach significance for rats ($t = 1.81$, $df = 18$, $P < 0.10$). This suggests that rearing may be a good reflection of the animals' exploration of the box, and that box exploration increased if there were no objects under the holes. Rearing generally showed significant habituation on trial 2 (see Table 2), and with objects present the rats had a mean score of 13.0 rears and without objects a mean of 11.4 rears. The mice made a mean of 38.3 rears with objects present and 40.5 rears without objects (in the absent-absent condition) and 50.2 rears in the present-absent condition. Only this last group of mice failed to show significant habituation, which again suggests that the mice were exploring the box itself more on trial 2 when objects were removed.

Table 1. Total head-dip scores

Species	Objects	Trial 1	Trial 2
Mice	present-present	29.4 ± 1.4	15.9 ± 3.3
	absent-absent	30.4 ± 3.3	26.8 ± 2.8
	present-absent	31.8 ± 1.5	24.9 ± 3.5
	absent-present	32.1 ± 2.6	30.8 ± 3.8
Rats	present-present	22.4 ± 2.8	13.8 ± 2.4
	absent-absent	17.6 ± 1.7	9.4 ± 1.5
	present-absent	17.8 ± 1.7	7.4 ± 1.8
	absent-present	18.4 ± 2.1	16.5 ± 2.8

The results are the means ± S.E.M. from 10 animals.

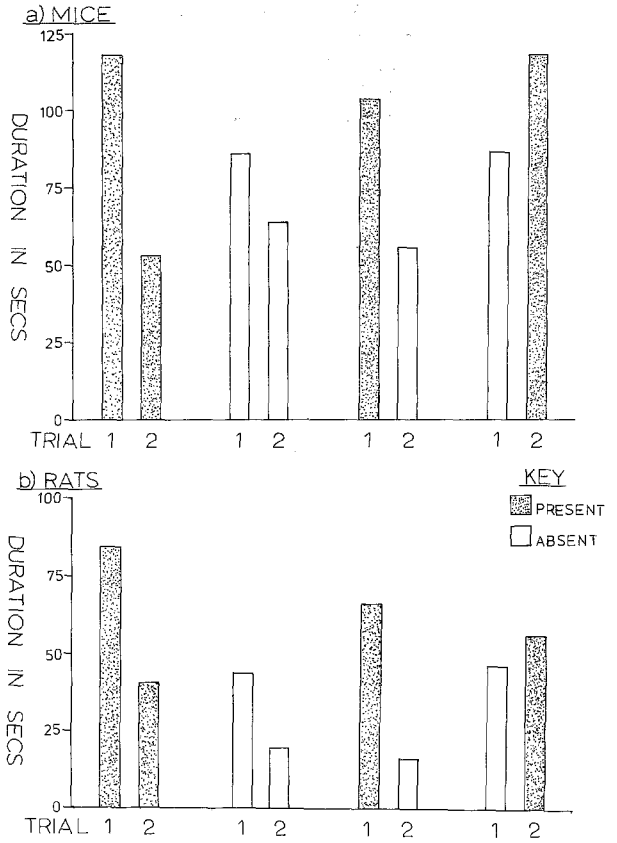

Fig. 1. Mean duration of head-dipping. Groups of 10 animals were tested twice in the presence or absence of objects

The mean defaecation scores for rats were 5.73 on trial 1 and 5.53 on trial 2; for mice they were 1.80 on trial 1 and significantly more, 2.98, on trial 2 (Wilcoxon $T = 151$, $P < 0.001$). Mice also showed significantly greater defaecation on trial 1 if there were objects under the holes (Mann-Whitney $U = 263$, $P < 0.05$).

For group E animals, an object was placed under one hole on trial 2. The data for the other three holes were combined and the means and standard errors

Table 2. Changes in total head-dips and duration of head-dipping and rearing over trials

Species	Objects	Total head-dips	Duration of head-dipping	Rearing
Mice	present-present	$t = 4.74$ $P < 0.01$	$t = 7.96$ $P < 0.001$	$t = 4.19$ $P < 0.01$
	absent-absent	$t = 2.09$ $P < 0.1$	$t = 3.49$ $P < 0.01$	$t = 2.36$ $P < 0.05$
	present-absent	$t = 2.78$ $P < 0.05$	$t = 7.32$ $P < 0.001$	$t = 1.49$ $P < 0.1$
	absent-present	$t = 0.40$ $P > 0.1$	$t = 2.71$ $P < 0.05$	$t = 6.38$ $P < 0.001$
Rats	present-present	$t = 3.75$ $P < 0.01$	$t = 4.10$ $P < 0.01$	$t = 1.89$ $P < 0.1$
	absent-absent	$t = 3.84$ $P < 0.01$	$t = 5.30$ $P < 0.001$	$t = 4.44$ $P < 0.01$
	present-absent	$t = 7.39$ $P < 0.001$	$t = 5.56$ $P < 0.001$	$t = 3.73$ $P < 0.01$
	absent-present	$t = 1.09$ $P > 0.1$	$t = 1.31$ $P > 0.1$	$t = 2.77$ $P < 0.05$

The changes from trial 1 to trial 2 were assessed by Student's 't'-tests. For all groups $n = 10$.

of total head-dips and duration for the two trials were calculated. The differences between the two trials were also assessed by related t-tests. These values were compared with those from the object absent-present hole (see Table 3). For the three holes which had no objects on either exposure, head-dipping decreased significantly. However, the frequency and duration of head-dipping either remained the same or increased on trial 2 at the hole where the object was placed. This indicates that the animals were paying particular attention to that hole.

Since this is a new piece of apparatus, its reliability was also considered. The test-retest reliability was assessed by calculating Pearson's product-moment correlation coefficients (see Table 4). High positive r-values were obtained in most cases, despite the low group numbers, which indicates the reliability of the apparatus.

Experiment 2

Method

Subjects. Thirty male CFW mice, 15–20 g in weight, and nineteen male hooded Lister rats, 250–315 g in weight, were used. They were housed and tested under the same conditions as in Experiment 1.

Apparatus. The hole-boards described in Experiment 1 were again used. The second mouse hole-board used was 40 cm square and 1.8 cm thick, with sixteen equally spaced holes in

Table 3. Total head-dips and duration of head-dipping for group E

Species	Objects	Total head-dips		Duration of head-dipping	
		Day 1	Day 2	Day 1	Day 2
Mice $n = 13$	absent-absent (3 holes)	6.0 ± 0.3	3.9 ± 0.4 $t = 5.79$, $P < 0.001$	16.4 ± 1.4	8.3 ± 0.7 $t = 6.58$, $P < 0.001$
	absent-present (1 hole)	6.0 ± 0.5	5.8 ± 0.9 $t = 0.21$, $P > 0.05$	17.1 ± 2.1	23.1 ± 4.9 $t = 1.24$, $P > 0.05$
Rats $n = 10$	absent-absent (3 holes)	2.4 ± 0.4	1.5 ± 0.3 $t = 2.65$, $P < 0.02$	3.8 ± 0.6	2.5 ± 0.6 $t = 2.03$, $P < 0.05$
	absent-present (1 hole)	1.8 ± 0.5	2.5 ± 0.6 $t = 1.02$, $P > 0.05$	3.3 ± 0.8	6.6 ± 1.5 $t = 2.06$, $P < 0.05$

In Group E objects were absent from all the holes on trial 1 but on trial 2 a single object was placed under one hole, there being no objects under the other 3 holes. The results are the means ± S.E.M. for 3 holes combined for the Absent-Absent condition, and the means ± S.E.M. for the one hole in the Absent-Present condition. The changes from day 1 to 2 were assessed by Student's 't'-tests.

Table 4. Test-retest correlations

Species	Objects	Total head-dips	Duration
Mice $n = 10$	present-present	$r = -0.07$	$r = 0.67$
	absent-absent	$r = 0.85$	$r = 0.88$
	present-absent	$r = 0.76$	$r = 0.40$
	absent-present	$r = 0.56$	$r = 0.73$
Rats $n = 10$	present-present	$r = 0.62$	$r = 0.60$
	absent-absent	$r = 0.12$	$r = 0.49$
	present-absent	$r = 0.67$	$r = 0.36$
	absent-present	$r = 0.77$	$r = 0.89$

All animals were given two trials in the hole-board, with objects present or absent. r = Pearson's Product-Moment correlation coefficient.

the floor, each 3 cm in diameter. It was mounted on four 25 cm legs and placed in a three-sided grey cubicle, with the observer seated at the fourth side. The second rat hole-board was of the same dimensions as the first, but with sixteen equally spaced holes in the floor.

Objects were not placed under the holes in the experiment.

Procedure. The procedure was generally the same as for Experiment 1, except that each animal was given a 5 min exposure to one hole-board, followed immediately by another 5 min exposure to the second. Half the animals were tested on the "four hole-board" first, and half on the "sixteen hole-board" first.

For each animal, the total number of head-dips made on each 5 min exposure was scored. The time spent head-dipping was not measured in this experiment, due to the high proportion of very brief dips obtained using the "sixteen hole-board", and the resultant difficulty in accurately measuring duration. Activity was also measured, by the amount of time each animal spent moving about on the hole-board, excluding the time spent grooming and rearing.

Results

The correlation between the head-dip and activity scores in the two hole-boards was assessed by calculating Pearson's product-moment correlation coefficients. The correlation between the total head-dip scores was: for mice $r = 0.22$ ($t = 1.19$, $df = 28$, $P > 0.05$); for rats $r = 0.59$ ($t = 2.97$, $df = 17$, $P < 0.01$). Thus head-dipping behaviour correlated significantly for rats, but not for mice.

The correlation between the activity scores was: for mice $r = 0.65$ ($t = 4.48$, $df = 28$, $P < 0.001$); for rats $r = 0.41$ ($t = 1.87$, $df = 17$, $P < 0.1$). Thus activity in the two hole-boards correlated significantly with mice, but just failed to reach significance with rats.

Experiment 3

Method

Subjects. Ninety male mice of the CFW strain, weighing between 15–20 g and 25–30 g, were used. They were housed and tested under the same conditions as in the previous experiments. Experimental groups comprised 15 animals.

Apparatus. The mouse "four hole-board" described in Experiment 1 was used, with the same 4 objects placed underneath the holes.

Procedure. (+)Amphetamine sulphate and pure alcohol were dissolved in saline and injected intraperitoneally at doses of 2 and 5 mg/kg and 0.4 and 0.8 g/kg respectively. The (+)amphetamine was injected in a volume of 0.2 ml/100 g body weight. A 15% alcohol solution was used and 0.1 ml given to 30 g mice for the 0.4 g/kg dose and 1.7 ml given to 25 g mice for the 0.8 g/kg dose. Control animals were injected with an appropriate volume of saline. (+)Amphetamine was injected 30 min before testing and alcohol 20 min before testing.

The procedure for testing was generally the same as in Experiment 1. Each animal was given a 10 min exposure to

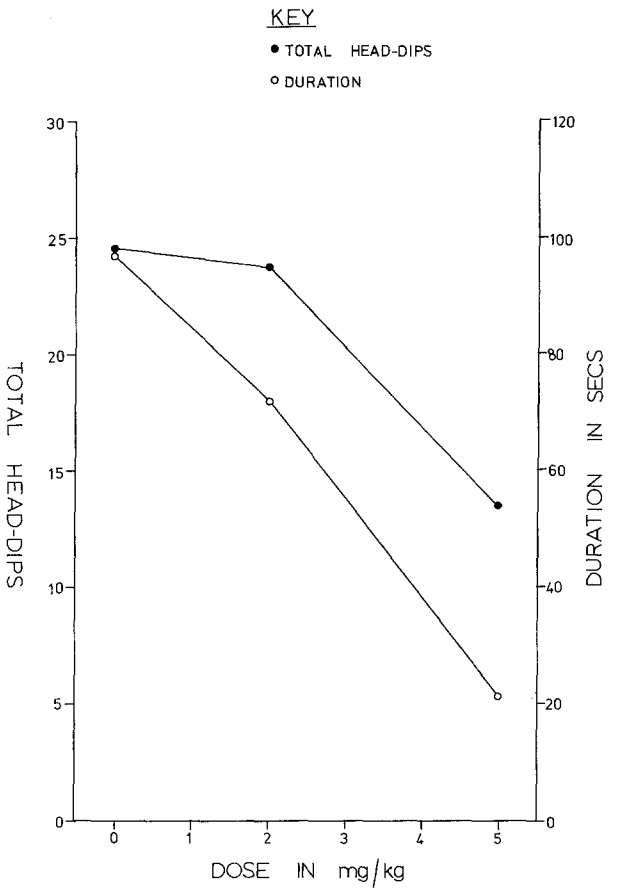

Fig. 2. Effects of (+)amphetamine on total head-dips and duration of head-dipping

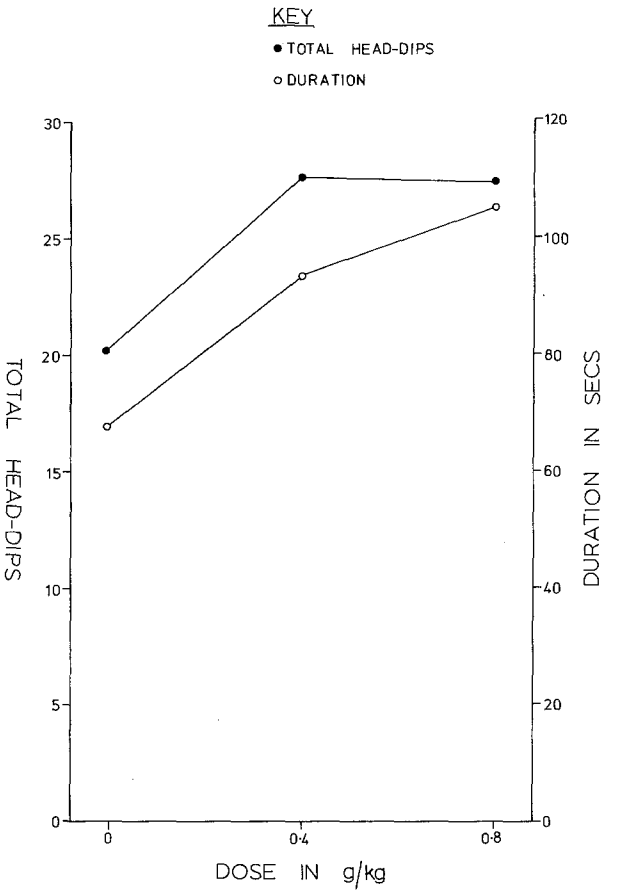

Fig. 3. Effects of alcohol on total head-dips and duration of head-dipping

the hole-board and the total head-dips, duration of head-dipping, rearing and defaecation scores were recorded.

Results

From Fig. 2, it can be seen that (+)amphetamine produced a decrease in both the frequency and duration of head-dipping. Analyses of variance confirmed significant drug effects and also significant linear trends for the total head-dips measure ($F = 6.39$, $df = 2,42$, $P < 0.01$ and $F = 10.38$, $df = 1,42$, $P < 0.01$ respectively) and for the duration measure ($F = 27.89$, $df = 2,42$, $P < 0.001$ and $F = 53.96$, $df = 1,42$, $P < 0.001$ respectively).

Although (+)amphetamine was observed to have a clear motor stimulant effect at the higher dose, rearing was not significantly changed. The mean rearing scores were: saline 63.3 ± 4.6; (+)amphetamine 2 mg/kg 53.7 ± 7.6 and 5 mg/kg 61.5 ± 7.9 ($F = 0.55$, $df = 2,42$, $P > 0.05$).

(+)Amphetamine produced a decrease in defaecation levels from a saline value of 1.3 to 0.3 with 2 mg/kg and 0.1 with 5 mg/kg. A Kruskall-Wallis non-parametric analysis of variance showed this drug effect to be significant ($H = 11.50$, $df = 2$, $P < 0.01$).

From Fig. 3, it can be seen that alcohol produced an increase in both the frequency and duration of head-dipping. Although the 0.8 g/kg dose did not increase total head-dips more than the 0.4 g/kg dose, the increase in duration was dose-dependent. The analyses of variance confirmed a significant drug effect and a significant linear trend for the total head-dips measure ($F = 4.91$, $df = 2,42$, $P < 0.05$ and $F = 7.23$, $df = 1,42$, $P < 0.05$ respectively) and for the duration measure ($F = 6.29$, $df = 2,42$, $P < 0.01$ and $F = 12.08$, $df = 1,42$, $P < 0.01$ respectively).

Alcohol produced a dose-dependent decrease in rearing scores from a saline mean value of 60.7 ± 7.5 to 54.7 ± 3.7 for the 0.4 g/kg dose and 46.7 ± 4.1 for the 0.8 g/kg dose. However, an analysis of variance showed that this effect was not significant ($F = 1.71$, $df = 2,42$, $P > 0.05$) and a test for linear trend just failed to reach significance ($F = 3.39$, $df = 1,42$, $P < 0.1$).

Alcohol produced an increase in defaecation levels from a saline value of 0.5 to 1.4 with 0.4 g/kg and 0.7

with 0.8 g/kg. A non-parametric analysis of variance showed this drug effect to be significant ($H = 6.98$, $df = 2$, $P < 0.05$). Despite the significant drug effects obtained on defaecation levels, it was felt that limited importance should be attached to these results due to the high percentage of animals showing zero defaecation levels (68.9% in the (+)amphetamine groups and 60.0% in the alcohol groups).

The duration scores for the two saline control groups were found to differ significantly ($t = 3.18$, $df = 28$, $P < 0.01$). This is due to the former group containing mice weighing between 15–20 g and the latter mice weighing either 25 g or 30 g, which emphasises the need to use animals of the same weight in control and experimental groups.

Discussion

In the introduction two criteria were proposed by which exploratory behaviour might be characterised. Firstly, if an animal is exploring, its behaviour should particularly reflect the novel aspects of the environment. It has been shown that when novel objects were placed underneath the holes on trial 1, the animals spent longer head-dipping. Also, when objects were introduced on the second exposure, the animals, instead of habituating as in the other conditions, actually spent longer looking down the holes than on the first exposure. This cannot have been due to a non-specific rise in arousal and activity due to the presence of the objects, because when only one object was introduced on trial 2, as with group E, the animals spent longer looking down this hole than the others. Also, the duration of head-dips at this hole increased over the two trials, whereas it decreased at the other three holes. Thus the animals' head-dipping behaviour reflected the attention paid to the novel objects. It might be argued that the change in environment produced by the removal of objects on trial 2 should have elicited increased head-dipping. However, the animals showed habituation under this condition. It could be that having explored both the objects and the holes on trial 1, one head-dip was sufficient to gain the information that the objects were missing, and thereafter the animals explored the holes less, because they had prior experience of them on trial 1.

When there was no change over the trials *i.e.* when there were objects present or absent on both exposures, all groups showed significant habituation. Thus, the second criterion proposed in the introduction, that exploration should result in information storage, has been satisfied. Although it is considered that these are necessary conditions for demonstrating exploration, it is recognised that they are probably not sufficient. However, within these limitations, the results suggest that head-dipping does reflect exploratory behaviour. Motor activity is involved in the measure of head-dipping only to the extent that the animal has to move to reach the holes, but it can do so slowly or with ataxia. The advantage of the measure is that it is not itself dependent on locomotion.

The results suggest that duration of head-dips is a better reflection of exploration than frequency, as the presence of objects was particularly indicated by the greater length of time the animals looked down the holes. All previous work on the hole-board has used a frequency measure, and it would in fact be very difficult to measure duration on the "sixteen hole-board" due to the high proportion of very brief dips which occur.

A positive correlation between frequency of head-dipping in the "four" and "sixteen" hole-boards was obtained for rats, but not for mice. This provides some indirect evidence that rats are exploring when head-dipping in the "sixteen hole-board", but no evidence for mice. It was the authors' subjective impression that mouse head-dipping in the "sixteen hole-board" is a more stereotyped behaviour, and that with such a high density of holes the mouse will almost compulsively dip at every opportunity. As activity correlated well in the two hole-boards for mice, it could be that mouse head-dipping in the "sixteen hole-board" is simply a good measure of motor activity. However, Simon *et al.* (1968) found that the frequency of head-dipping was increased in the sixteen-hole-board by introducing a warm air blast, and this suggests that mouse head-dipping in this apparatus may at least partly reflect exploration.

Existing data on the effects of amphetamine on behaviour in a novel environment are conflicting (Wimer and Fuller, 1965) but this is at least partly due to the different types of apparatus used. In those experiments (Wimer and Fuller, 1965; Kumar, 1969; Robbins and Iversen, 1973) where the measure of exploration was not heavily dependent on the level of motor activity amphetamine caused a decrease in exploration, and the results of Experiment 3 are consistent with this. However, Boissier and Simon (1964) found that 2.5–5 mg/kg (+)amphetamine increased the frequency of head-dipping in mice in the sixteen-hole-board. This result would be consistent with considering mouse head-dipping in the sixteen-hole-board as largely reflecting activity. Clearly this is at variance with the interpretation of the Boissier and Simon (1968) results and thus the interpretation of head dipping in the sixteen hole-board is still somewhat equivocal. There is less evidence on the effects of alcohol on exploration but 0.5 and 1.0 g/kg alcohol increased head-dipping in mice (Joyce *et al.*, 1972) and Steele (personal communication) found

0.4 g/kg increased exploration in rats, measured by head-poking for light reinforcement. The effects of alcohol in the four hole-board are consistent with low doses leading to increased exploration. Thus the effects of the drugs tested are consistent with viewing head-dipping in the four-holeboard as a measure of exploration and provide another source of validation for the apparatus.

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