A novel test of visual learning in the rat: effects of 8-OH-DPAT and WAY-100579

H. J. Cassaday¹ and E. A. Gaffan²

¹Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD and
²Department of Psychology, University of Reading, Reading RG6 6AL, UK

The 5-HT₁₄ agonist 8-OH-DPAT and the 5-HT₃ antagonist WAY-100579 were tested in a novel computerized visual learning task. Since rats show substantial learning of new problems within each single session, drug effects on new learning could be compared within subjects. Both the reaction time (RT) and choice accuracy were measured. A dose of 0.3 mg/kg of 8-OH-DPAT lengthened the RTs while 0.1 mg/kg of 8-OH-DPAT and 0.1 mg/kg of WAY-100579 shortened the RTs. In the absence of drugs, accuracy was independent of the RT for RTs > 1.5 s. Therefore, in order to unconfound the drug effects on accuracy from motor effects, only responses with longer RTs were analysed. Both 0.3 and 0.1 mg/kg of 8-OH-DPAT and 0.1 mg/kg of WAY-100579 significantly impaired accuracy, though some learning was seen in all cases. These findings may have implications for animal models of Alzheimer's disease.

Key words: visual learning; rat; 8-OH-DPAT; WAY-100579; Alzheimer's disease

Introduction

There is currently considerable interest in the serotonergic system from a range of clinical research perspectives, including aggression, anxiety, dementia, depression, eating disorders and schizophrenia. With the continuing identification of different receptor subtypes there is no necessary contradiction in the many functions attributed to this system (Humphrey, Hartig and Hoyer, 1993; Leonard, 1994). However, despite evidence that serotonin (5-HT) is involved in the cognitive changes associated with dementia (Altman and Normile, 1988; McIntee and Crook, 1991) and the widespread prescription of serotonergic compounds for affective disorders, there has been relatively little systematic investigation of the cognitive effects of serotonergic drugs.

Interest in the role of particular neurotransmitter systems in pathology is typically driven by post-mortem evidence of changes correlated with the disease process together with knowledge of the mechanism(s) of action of established drug treatments. In the case of Alzheimer's disease (AD), there are no effective drug treatments as yet. The concentration of research effort on the neurodegeneration of cholinergic pathways associated with AD has not resulted in effective cholinergic drugs to improve cognition (see Collerton, 1986; Palmer and Bowen, 1991). Attention has therefore shifted to other candidate neurotransmitters, including the serotonergic system (see Palmer and Bowen, 1991) and to relating neurochemical disturbance to the histopathological changes characteristic of AD. Specifically, 5-HT₁₄ receptors are present on the cortical pyramidal neurones which degenerate in AD and since neuronal activity should be enhanced by blocking the hyperpolarizing action of endogenous 5-HT on these cells, it may be predicted that 5-HT₁₄ antagonists should enhance or at least restore the functioning of the remaining pyramidal cells (Bowen et al., 1994). Conversely, agonists at the 5-HT₁₄ site, such as 8-OH-DPAT, may produce cognitive deficits in normal animals. However, the evidence on the latter point is at present contradictory.

The effective use of animal models requires careful analysis of the psychological processes engaged by the task and any non-cognitive effects of the experimental treatment, such as motor impairment, which may confound interpretation (see Sarter, Hagan and Dudchenko, 1992). The effects of the systemic administration of the 5-HT₁₄ agonist 8-OH-DPAT on working memory have been investigated using delayed matching and non-matching to position (Deacon, 1991; Cole, Jones and Turner, 1994; Stanhope et al., 1994a; Stanhope, McLenachan and Dourish, in press). Systemic 8-OH-DPAT administration has been reported both to reduce (Deacon, 1991) and to improve choice accuracy at low doses (Cole, Jones and Turner, 1994). There is agreement that the predicted reduction in cognitive function (see above) is difficult to dissociate from motor effects at the higher doses required to demonstrate such impairment (Cole, Jones and Turner, 1994; Stanhope et al., 1994a; Stanhope, McLenachan and Dourish, in press).

Here we use a new computerized Y-maze apparatus to assess discrimination learning with visual stimuli in the rat (Gaffan and Eacott, 1995). The task is one in which trained rats show substantial within-session learning of discriminations which are new in every session, allowing the effects of different drug treatments on new learning to be compared repeatedly within subjects by varying the treatment across sessions. The primary intention of the present study was to determine whether cognitive and motor effects could be
reliably dissociated in this apparatus, using a treatment of contemporary interest: systemic 8-OH-DPAT.

Effective pre-clinical screening ultimately requires that the paradigms adopted are sensitive to both improvements and impairments in cognitive function. The availability of selective 5-HT₃ antagonists, reported to reverse the cognitive deficits produced by treatment with scopolamine (Costall et al., 1989; Carey et al., 1992) and cholinergic lesions (Hodges et al., in press), has prompted considerable interest in their potential as 'cognitive enhancers'. We selected the 5-HT₃ antagonist, WAY-100579, to see whether bidirectional effects on performance could be detected with our new methods. Although the efficacy of 5-HT₃ antagonists is typically demonstrated in animal models of cholinergic deficiency (e.g. scopolamine injection) there is some evidence to suggest that they may also enhance cognitive ability in the normal animal (Domeney et al., 1991). Such enhancements would be consistent with the evidence that 5-HT₃ antagonists facilitate long-term potentiation in the hippocampus (Maeda, Kaneko and Satoh, 1994; Passani et al., 1994). Rats in the present study reacted so badly to scopolamine that its use was not an option, but since our task shares common elements with that used by Domeney et al. (1991), we went on to investigate the effects of WAY-100579 in normal animals.

The new procedure we used in the automated Y-maze is particularly suitable for testing the effects of drugs on learning and memory because, as previously mentioned, rats show rapid learning of each new discrimination problem, even when two or three new problems are given per day. The procedure therefore resembles the learning set paradigm of Harlow (1949). By the time this study commenced the subjects had much experience of it and had reached a stable baseline of learning rate within problems. One feature of the task, which we believe favours the rapid visual learning seen within problems, is that the stimuli are 'panoramic', extending across more than 90° of the horizontal visual angle and located some distance from the rat. A second feature is that the training procedure exploits rats' preference for relatively novel stimuli over familiar ones, as exemplified in their superior performance of non-matching to sample compared to matching (Aggleton, 1985). Each problem had a constant stimulus which appeared in every trial, paired with a trial-unique stimulus which was different in every trial. Choice of the variable, trial-unique stimulus was rewarded, so the procedure is known as constant-negative (see Gaffan and Woolmore, in press). Although a single daily problem could have been given, the averaging of data over two or more problems counteracts the variability produced by random differences in the attractiveness of particular constant stimuli. We were able to replicate and compare the effects of different pharmacological treatments on the learning rate within the same trained group of rats. The reaction times (RTs) were recorded on every trial, allowing us to detect motor changes and to dissociate the drug effects on response speed and accuracy (see the Results).

In experiment 1a, every session comprised three such problems, each involving a different constant and different set of variable stimuli; 20 choice trials were given within each problem. In experiments 1b and 2, two problems were given per session, each with 30 choice trials. The stimuli used for each session's problems were freshly randomized daily.

Experiment 1a examined the effect of two doses of 8-OH-DPAT, 0.1 and 0.3 mg/kg, on visual learning. Whilst these doses are within the ranges used which are reported to produce motor effects in tests of working memory (Deacon, 1991; Stanhope et al., 1994a,b; Stanhope, McLenachan and Dourish, in press), the use of relatively high doses of 8-OH-DPAT may be necessary to demonstrate cognitive effects since there is evidence that such effects are post-synaptically mediated (Carli and Samanin, 1992). However, our lower dose also corresponds to that reported to improve working memory in normal rats (Cole, Jones and Turner, 1994). Experiment 1b was a second replication of the same drug treatments after the rats had received further training on the task. In experiment 2, the effect of 0.3 mg/kg of 8-OH-DPAT was retested and also that of the 5-HT₃ antagonist WAY-100579.

Methods

Experiment 1a

Subjects

Eight Dark Agouti (DA) male rats (Harlan-Olac, Bicester, Oxon) aged 9 months, were housed in pairs and fed so as to remain at or above 85% of the free-feeding weight. All had extensive previous training with the constant-negative procedure. Their previous training had employed one of two classes of stimuli, 'scenes' or 'objects', which could be stationary or moving (see Apparatus and stimuli below for details). Five had been trained throughout with scene stimuli and three with objects and they continued with the same stimulus class here. Their performance with stationary and moving stimuli had previously been compared (Gaffan and Woolmore, in press) and, in the present study, each rat was tested with either all moving or all stationary stimuli, depending which had generated better performance hitherto (although the differences were not great). Three of the five scene rats were given moving stimuli and so were all the three object rats.

Apparatus and stimuli

The apparatus was a computer-controlled Y-maze (Gaffan and Eacott, 1995). It had three arms, 17 cm wide at the entrance, broadening to 50 cm immediately in front of two adjacent monitor screens (30 cm diagonal) at the end of each arm, approximately 40 cm from the maze centre. From the maze centre where rats made their choices, all the stimuli could be seen. At the end of each of the arms, food dispensers delivered 45mg diet pellets (BioServ) to food trays in the narrow spaces between the adjacent screens. A light within each food tray indicated when pellets were available and a hinged perspex flap across the tray aperture was fitted with a microswitch to detect the rat's entry to the tray. The rats' locations in the maze were detected by two infrared photodetector beams extending across each arm, 23 cm and 38 cm from the maze centre. They could move freely round the maze and there were no doors. The threshold of each arm had a low (1 cm) metal barrier; however, arm entry was defined by the rat interrupting the photobeam nearer the centre.

The monochromatic stimuli were of two kinds, scenes and objects. All stimuli extended across two screens and were left-right symmetrical. Scenes consisted of backgrounds made up
Table 1 Procedure for Constant-Negative procedure: stimuli change randomly from one problem to the next

<table>
<thead>
<tr>
<th>Expt 1a Constant-Negative Procedure: 3 problems per session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 1</td>
</tr>
<tr>
<td>Problem 1: 01+01 — 01 — 01 — 01 — 02 + 03 + 01 — 04 + 01 — 01 — 05 + ... — 01 — 21 +</td>
</tr>
<tr>
<td>Problem 2: 22 — 22 + 22 — 22 — 22 — 23 + 22 — 24 + 22 — 25 + 26 — 22 — ... — 42 + 22 —</td>
</tr>
<tr>
<td>Problem 3: 43 — 43 + 43 — 43 — 45 + 43 — 46 + 43 — 43 — 47 + 43 — 48 + ... — 63 + 43 —</td>
</tr>
<tr>
<td>Session 2</td>
</tr>
<tr>
<td>Problem 2: 60 + 60 — 60 — 60 — 60 + 60 — ... — 60 — 60 — 60 — 22 + 34 — 60 — 16 + ... — 21 + 60 —</td>
</tr>
</tbody>
</table>

01, 02 etc are individual stimuli from the 70 available.
01, 22, 43, 15, 60, 39 are grey fields matched in brightness to stimuli 01, 22, 43, 15, 60, 39.
+/- mean that approaching stimulus is rewarded/not rewarded.
For each problem within a session, 21 different stimuli are used, one constant and 20 trial-unique.
Each session, 63 stimuli are randomly chosen and ordered for the 3 problems.
In Session 1, the Constant stimuli are 01, 22 and 43.
In Session 2, the Constant stimuli are 15, 60 and 39.

of either dark or light greys, uniform or slightly varied, with a number of smaller shapes of contrasting brightness distributed across the backgrounds. Objects were large single shapes, centrally placed on the pair of screens, varying in height and width but extending no further than approximately half way across each screen. They were all displayed against a black background and could be plain or patterned. Any stimulus could be displayed either stationary or moving in oscillatory fashion. The stimulus pool comprised 70 different scenes and 70 different objects. For examples see Gaffan and Eacott (1995).

Procedure
There were four to five training sessions per week, normally lasting 15–20 min. Each session comprised three problems. Each problem employed 21 different stimuli, one constant and 20 variables. The 63 different stimuli required were freshly randomized each session from the 70 available, independently for each rat. As described above, the constant stimulus was presented on every trial of a problem, but only rewarded at its first appearance; thereafter, the rat was rewarded for choosing variable (relatively novel) stimuli in preference to the constant (see Table 1).

For each trial, one maze arm was defined as the start arm, the remaining two being the choice arms. When the rat entered the start arm, the two choice stimuli were made visible in the other arms. The left–right position of the choice stimuli relative to the start arm was randomized throughout.

For the first two trials of a problem, the choice was between the constant stimulus in one arm and a brightness-matched uniform grey. The purpose was to pre-expose the rats to the constant prior to the main discrimination test trials. Both stimuli remained until the rat entered the arm containing the constant. On the first trial, approaching the constant was rewarded with one 45 mg food pellet; the constant remained on screen, the tray light came on and both remained until the rat had pushed the tray flap to collect the pellet. This arm then became the start arm for the next trial. Subject to a check that the rat was still in that arm (i.e. one of the two photobeams was interrupted) the same two stimuli were then immediately presented in the other two arms. In the second trial, when the constant was approached, it remained on screen and the tray light came on for 3s, but no pellet was dispensed: this contingency conformed to the general rule that the constant was rewarded only on its first appearance in the session.

The remaining 20 trials offered a choice between the constant and a variable stimulus. A new variable was used in every trial, i.e. the variables were trial unique. In these trials, if the rat approached the constant, both stimuli terminated without reward. If it approached the variable, two reward pellets were separately dispensed over a 5 s period while the tray light and variable stimulus remained on. When the second pellet had been collected, both stimuli were terminated. The RT from stimulus onset to arm entry was measured in centiseconds (sec), for all choices whether correct or wrong.

Whichever arm the rat had entered became the start arm for the following trial. If it had chosen correctly, the next pair of choice stimuli were presented immediately, subject as usual to a check that the rat was in the start arm. If it had made an error, a further 2 s delay was imposed. At the end of that delay, the rat was required to be in the new start arm before the next trial could commence; it had moved out of that arm during the delay, the programme waited until it returned there. After the twentieth choice trial, there was a 12 s interval before the next problem started. Baseline sessions without injections were run on most but not all of the days between treatments (see below) and on 3 days preceding and 4 days following the main study, amounting to 15–17 'no-injection' sessions per rat.

Treatments
8-OH-DPAT (Sigma) was warmed in a saline vehicle to produce a solution and administered by i.p. injection 15 min before testing. The doses were calculated as the weight of the salt. Control injections of vehicle (saline) were also given 15 min before testing. The injection volume in each case was 1 ml/kg.

During a 3 week period, injections were given before test sessions on 2 days per week (usually Tuesday and Friday). Each rat was tested twice with 8-OH-DPAT at 0.1 mg/kg, twice with 8-OH-DPAT at 0.3 mg/kg and twice with saline vehicle, following a Latin square design.
Statistics
All statistical tests employed a $\alpha$ of 0.05, with corrections for deviations from sphericity in repeated-measures ANOVAs (Huynh and Feldt, 1976). Omnibus ANOVAs on the effect of the drug treatments — no injection, saline and doses of 8-OH-DPAT — were followed by selected comparisons between each of the latter three conditions and no-injection, employing separate error terms (see Hertzog and Rovine, 1985; Vasey and Thayer, 1987). Figures show pooled SEs for the main effects of drug, where appropriate.

Experiment 1b
Subjects
They were the same eight rats who had taken part in experiment 1a, maintained as before. They resumed baseline training (without injections) 6 weeks after the end of the previous study, aged approximately 12 months. This retraining lasted 8 weeks, so their age at the start of the experiment proper was 14 months.

Apparatus and stimuli
These were the same as for experiment 1a, except that only moving scene stimuli were employed (see Procedure: extended no-injection training).

Procedure: extended no-injection training
During 33–35 sessions of further training on the constant-negative procedure, various manipulations were tried in order to reduce the number of short RT choices and, partly as a consequence, to raise the performance overall to a higher level. Some procedural changes (e.g. reducing the reward magnitude for short-latency choices which were correct) had little effect and were discontinued. The changes which appeared beneficial and were maintained during later drug testing, were as follows. Only two problems were given per session, each with 30 choices; all rats were now trained with scenes, as the rats given scenes in experiment 1a had performed better than those given objects and all were trained with moving stimuli, because observation suggested that the need to sample the movement pattern for a short time naturally encouraged longer latency choices.

Replication of testing with 8-OH-DPAT
The drug treatment procedures were identical in experiments 1a and 1b, but with the changes in the basic training schedule described above and the following treatment schedule. There were two injection tests per week interspersed with no-injection sessions and data were also recorded from three no-injection sessions preceding the first drug test and two no-injection sessions following the last. The saline condition and the two doses of 8-OH-DPAT were again each tested twice and the no-injection condition 11 or 12 times. The statistical procedures were the same as those described for experiment 1a.

Experiment 2
We finally used the same methods to examine the effect of the 5-HT$_3$ antagonist WAY-100579. 5-HT$_3$ antagonists have been reported to improve normal performance in T-maze alternation (Barnes et al., 1990) and object discrimination tasks (Domeney et al., 1991), but have no effect on normal rats' spatial learning in the water maze (Rogers, Olufsen and Piper, 1994). In common with other 5-HT$_3$ antagonists, WAY-100579 is capable of reversing impairments in spatial learning induced by cholinergic lesion (Hodges et al., in press). The effects of 5-HT$_3$ antagonists, such as ondansetron, injected alone in normal animals, are more controversial but may be seen in the same dose range required to reverse the effects of scopolamine (Domeney et al., 1991; Carey et al., 1992). Since Domeney et al. (1991) used a different 5-HT$_3$ antagonist and marmosets rather than rats, we selected the dose of WAY-100579 found most effective by Hodges et al. (in press). In addition to testing 0.1 mg/kg of WAY-100579, we retested the effect of 8-OH-DPAT at 0.3 mg/kg.

Subjects, apparatus and stimuli
These were the same as in experiment 1b. The rats were aged 15 months.

Procedure
The experiment commenced immediately after the end of experiment 1b. Two additional no-injection sessions were run before and after the test sessions (see below), making 10 no-injection sessions in all.

Treatments
Treatment with 0.3 mg/kg 8-OH-DPAT (Sigma) was identical to that described for experiment 1a. WAY-100579 (Wyeth Research, UK) was dissolved in a saline vehicle and administered by i.p. injection 30 min before testing. Control injections of vehicle (saline) were given 15 and 30 min before testing.

Over a 3 week period, six drug treatment sessions were given, interspersed with no-injection sessions. There were two replications each of saline, 8-OH-DPAT (0.3 mg/kg) and WAY-100579 (0.1 mg/kg). One saline injection was given at each of the two pre-test intervals.

Statistics
Omnibus ANOVAs on the effect of drug treatments — no-injection, saline, 8-OH-DPAT and WAY-100579 — were followed by selected comparisons between each of the latter three conditions and no-injection. The statistical procedures were identical to those described for experiment 1a.

Results
Experiment 1a
We examined both the RTs (as an indication of the motor or motivational effects of the drug) and the proportion of correct choices. We recognize that these measures may be interdependent and their relationship is discussed below.

Figure 1 shows, on the left, the per cent of correct choices averaged across all problems learned after each drug treatment, i.e. 45–51 problems (15–17 sessions) in the no-injection condition and six problems (two sessions) in each drug condition. The 20 trials per problem are divided into two 10 trial blocks.
A NOVEL TEST OF VISUAL LEARNING IN THE RAT

Figure 1  Effects of 8-OH-DPAT (0.1 and 0.3 mg/kg) on the accuracy of visual learning and response latency in experiment 1a

From the raw data it appears that both doses of 8-OH-DPAT impaired performance, but note that the two doses had very different effects upon the choice latency, shown on the right of Fig. 1. Choice RT speeded up under 0.1 mg/kg of 8-OH-DPAT, but slowed under 0.3 mg/kg. The effect of the higher dose was very variable across rats, some showing little obvious effect while others slowed dramatically. A conventional ANOVA was not applied to the latency data because of the heterogeneity of variance. Selected comparisons of each drug dose with no-injection showed that the effect of saline was non-significant \( F < 1 \), the acceleration at 0.1 mg/kg was significant \( F(1,7) = 19.2 \) and the slowing under 0.3 mg/kg was not \( F(1,7) = 1.94 \), because of its variability.

Inspection of the data from no-injection sessions suggested a positive association between accuracy and RT. In sessions in which the mean accuracy was better, the mean RT was longer, the correlation being statistically significant within the no-injection data of every individual rat. The relationship between RT and choice accuracy means that, because 8-OH-DPAT affected RT, its apparent effects on the accuracy cannot be taken at face value. We therefore divided trials into short, medium and long RT categories, in order to compute the mean accuracy separately for each category.

The three ‘bands’ of RT were defined by preliminary examination of each rat’s distribution. The ranges selected were 0–150 csec, 151–300 csec and 301 csec upwards. These were chosen because, in no-injection sessions, responses were on average equally distributed between them and because every rat made some choices within each band in every drug condition. Figure 2A shows the percentage of choices whose latencies fell within each RT band, separately for the no-injection sessions and under the three drug conditions. Consistent with the pattern of mean latencies shown in Fig. 1, saline had no effect and the lower dose of 8-OH-DPAT resulted in relatively more short and medium RTs and fewer longer ones, while the higher dose had the converse effect (Fig. 2A). The scores (proportions of choices) were subjected to ANOVA with the drug and RT band as factors. The interaction was significant \( F(6,42) = 5.38 \). Analysis of the simple effects confirmed the differences between the drug conditions in each of the RT bands \( F(3,21) = 3.26, 9.30 \) and 5.38 respectively. Selected contrasts relative to no-injection showed that 0.1 mg/kg of 8-OH-DPAT reliably increased the proportions of short and medium RTs and reduced the proportion of long RTs. However, the increase in long RTs under the 0.3 mg/kg dose was again too variable to attain significance by this measure.

We then computed the proportion of choices which were correct, in each separate RT band (see Fig. 2B). In the absence of the drug, choices made with short RTs were less likely to be correct than those made at longer RTs. The difference between the RT bands was significant for both the no-injection and saline conditions \( F(2,14) = 5.39 \) and 6.33; in both conditions the scores were significantly lower for short RT choices than for others. This analysis confirms that, in normal rats, rapid responses are less accurate than slower ones. Moreover, the similar performance for all RTs greater than 150 csec implies that the correlation between latency and accuracy (described
Experiment 1b

Figure 3 Effects of 8-OH-DPAT (0.1 and 0.3 mg/kg) on the accuracy of visual learning in experiments 1a and 1b, excluding short RT choices. Bars show the SE for the main effects of drug, within each experiment.

above) can be accounted for by differences in the proportion of short RT choices.

It is also clear that, for responses made with short RTs, accuracy was unaffected by the drug, while in both longer RT bands the drug tended to impair discrimination. Statistical analysis confirmed that the drug conditions had no effect on the accuracy of responses in the shortest RT band ($F < 1$). Preliminary analysis (a drug × RT band ANOVA) revealed no statistical difference between the pattern of drug effects in the two longer RT bands. We decided therefore that the effect of the drug could be most sensitively assessed by eliminating the short RT choices, where discrimination was both weak and impervious to the drug and pooling all longer RT choices for analysis.

In essence this procedure allows us to unconfound the effect of a dose of the drug upon the discrimination accuracy from its effect on the RT distribution. The unconfounding is possible because in the no-injection and saline conditions the accuracy was constant for all RTs over 150 sec; thus, by including only responses whose RTs exceeded 150 sec, we ensure that any drug effects on accuracy are not secondary to effects on RT.

Figure 3 (left side) shows the mean percent correct in 10 trial blocks after deletion of all trials in which the choice latency was $\leq 150$ sec. From Fig. 2(A) it can be seen that this entailed omitting on average 30% of the trials from the no-injection and saline conditions, 33% of the 0.1 mg/kg sessions and 20% from the 0.3 mg/kg sessions. The deletion was not selective from the first and second 10 trial blocks.

Because the long RT choices were relatively accurate, the overall percent correct is higher than in Fig. 1, but otherwise follows a similar pattern. An ANOVA yielded significant effects of blocks [$F(1,7) = 15.78$] and drug [$F(3,21) = 4.11$] conditions but no interaction ($F < 1$). Selected contrasts against the no-injection condition showed that saline injections had no effect ($F < 1$), but both doses of 8-OH-DPAT impaired accuracy [0.1 mg/kg, $F(1,7) = 30.26$ and 0.3 mg/kg, $F(1,7) = 9.43$].

Experiment 1b

Figure 4(A) shows the proportions of choices which fell into the previously defined RT bands, under all four conditions of the final drug testing phase, in the same format as Fig. 2(A). The overall proportion of short RTs was approximately 20%, much lower than in experiment 1a; for example in the no-injection condition, the decrease was shown by every rat and was significant [$F(1,1) = 34.3$]. The pattern of effects of the drug was similar to that seen previously, with the 0.1 mg/kg dose causing a shift towards shorter RTs and the 0.3 mg/kg dose producing relatively longer RTs. The drug × band interaction was significant, [$F(6,42) = 20.3$] and simple effects analysis confirmed that the changes produced by both doses in the medium and long RT bands were significant.

The accuracy of choice within each RT band (Fig. 4B) followed a similar pattern to the previous experiment (cf. Fig. 2B). Short RT choices were consistently less accurate than others, the two longer RT bands being similar. In the no-injection condition, there was a significant effect of band [$F(2,14) = 18.45$], with both longer RT bands yielding significantly higher scores than the short RT band [$F(1,7) = 22.71$ and 41.30]. Thus, any effect of the RT on discrimination stemmed entirely from the inaccurate short RT responses.

As for drug effects, they were again similar to the previous experiment though less clear-cut. Although inspection suggests that 0.3 mg/kg of 8-OH-DPAT impaired the choice accuracy in all RT bands, the drug effect was not significant overall [$F(3,18) = 2.39$], nor was the drug × band interaction ($F < 1$). A test confined to the short RT band, where there had been no drug effect in experiment 1a, confirmed that the same was true here also [$F(3,18) = 1.15$]; the larger dose of 8-OH-DPAT produced a low average score, but the data were very variable. The drug effects in the longer RT bands, though small, were qualitatively similar to those obtained before.
The right-hand side of Fig. 3 shows the results of experiment 1b for direct comparison with the first replication, with short RT choices excluded; there are three 10 trial blocks because each problem comprised 30 choice trials. Inspection of the means suggests that, unlike experiment 1a, saline injection here produced some initial impairment. However, the specific comparisons applied to experiment 1b showed no significant difference between the saline and no-injection conditions \( F(1,7) = 1.71 \), while both 0.1 and 0.3 mg/kg of 8-OH-DPAT significantly reduced the accuracy below the no-injection condition \( F(1,7) = 6.24 \) and 70.8. Thus, discarding the short RT responses again seems to reveal drug effects more clearly.

The two replications were analysed together in a replications \( \times \) drug \( \times \) blocks ANOVA, confined to the first two 10 trial blocks. The effect of replications was significant \( F(1,7) = 9.10 \), reflecting the generally better performance in the second replication, but the replication factor did not interact significantly with any other. There were significant effects of drug \( F(3,21) = 4.77 \) and block \( F(1,7) = 19.39 \), but no drug \( \times \) block interaction \( F < 1 \). Contrasts with no-injection showed no effect of saline \( F < 1 \), but significant impairment by both lower and higher doses of 8-OH-DPAT \( F(1,7) = 7.16 \) and 28.45. In experiment 1b, unlike experiment 1a, there was some sign that saline injections impaired performance. Although this was not a significant effect, and the saline data provide a less reliable baseline because it was derived from only four replications across the two experiments, we additionally compared the two doses of 8-OH-DPAT to the saline condition. The 0.3 mg/kg dose produced a significant impairment relative to saline \( F(1,7) = 6.42 \), while the 0.1 mg/kg dose did not \( F(1,7) = 1.02 \), confirming the suggestion of a dose–response relationship in Fig. 3.

**Experiment 2**

Figure 5 shows the proportions of RTs in the three bands and the percentage correct choices within each band. From Fig. 5(A), it is clear that 8-OH-DPAT at 0.3 mg/kg once again slowed responding, but WAY-100579 tended to speed it up, with a shift towards shorter RTs. The drug \( \times \) RT band interaction was significant \( F(6,42) = 5.42 \). The effect of WAY-100579, while not large, was significant; relative to no-injection it produced more medium RT and fewer long RT choices \( F(1,7) = 6.21 \) and 10.43. The slowing produced by 8-OH-DPAT was variable and of marginal significance \( F(1,7) = 4.68, p = 0.07 \) on short RTs and \( F(1,7) = 5.52, p = 0.051 \) on long RTs.

The accuracy of discrimination in different RT bands is shown in Fig. 5(B). Once again the bands differed in the no-injection condition \( F(2,14) = 9.46 \), because short RT responses were significantly less accurate than those in either of the longer bands \( F(1,7) = 7.96 \) and 19.26. Moreover, the accuracy of short RT responses was unaffected by the drug \( F < 1 \). Thus, the main analysis of the drug effects was carried out after discarding short RT data, for the same reasons as before.

Figure 6 shows the discrimination accuracy in 10 trial blocks within problems, omitting data from trials with response latency \( < 150 \) msec, averaged over 10 no-injection sessions (20 problems) and two sessions (four problems) under each drug condition. Both 8-OH-DPAT and WAY-100579 depressed accuracy particularly in later blocks. Here, as well as significant effects of drug \( F(3,21) = 4.01 \) and blocks \( F(2,14) = 16.38 \), there was a significant interaction \( F(6,42) = 3.13 \). Analysis of interaction contrasts revealed that the interaction stemmed from the saline condition, not from either drug condition. Saline injections resulted in poor scores in early blocks, but did not prevent discrimination reaching high levels later. A similar, non-significant pattern was visible in experiment 1b though not experiment 1a (see Fig. 3). The saline treatment did not impair the accuracy overall relative to the no-injection condition \( F < 1 \), whereas both 8-OH-DPAT and WAY-100579 did so. The effect of 8-OH-DPAT \( F(1,7) = 6.06 \) was of similar magnitude to that seen previously. The impairment produced by WAY-100579, while smaller, was reliable \( F(1,7) = 10.43 \).
Discussion

Experiment 1a

Doses of 8-OH-DPAT with directly opposite effects on latency tended to impair accuracy in the visual learning task. The accuracy of rapid choices (with RTs below 1.5s) was insensitive to the effects of 8-OH-DPAT so drug effects were further assessed after excluding the short-latency choices from the analysis. This clearly confirmed that both doses of 8-OH-DPAT impaired accuracy on the visual learning task (Fig. 3).

The relationship between RT and accuracy could, in theory, have taken a number of forms; it could have been negative or positive and the causal relation could work in either direction. Excessively fast or slow reactions may result in poor discrimination or, alternatively, an animal who has difficulty in discriminating might respond stereotypically (causing faster reactions) or hesitantly (causing slower reactions) as a result. In fact the correlation was positive. An informal observation of the rats suggested that, in some trials, they appeared to choose in a rapid 'stereotyped' manner, moving to the left or right without inspecting the choice stimuli.

The 0.1 mg/kg dose both speeded up the response, increasing the proportion of shorter latency choices and impaired accuracy. At first sight one might be tempted to interpret the second effect as a side-effect of the first. However, Fig. 2(B) shows that the effect on accuracy is separable from the effect on response speed since the accuracy was reduced most by 0.1 mg/kg 8-OH-DPAT in the longest RT band. If this dose of
the drug affected only speed (Fig. 2A), the accuracies in each RT band should have resembled those obtained in the no-injection and saline conditions (Fig. 2B), but they did not.

The 0.3 mg/kg dose differed greatly from the 0.1 mg/kg dose in terms of its effect on speed, but its effect on accuracy was rather similar, although a small dose–response effect is visible in Fig. 3.

These methods are therefore capable of dissociating the 'motor' effects of the drug from effects on rapid visual learning, if the RT is measured and used as a criterion for rejecting some of the data. There are obvious disadvantages to this procedure. We therefore gave the rats further training with the aim of reducing the number of short-latency choices, allowing more of the data to be retained for analysis. Following this further training, experiment 1b retested the effects of the same drug treatments.

**Experiment 1b**

The separate and combined analyses of experiments 1a and 1b confirmed that both 0.1 and 0.3 mg/kg of 8-OH-DPAT impaired the rats' accuracy in the rapid visual learning task, despite having very different motor effects as reflected by their opposite effects on response latency. The analysis of per cent correct separately for different ranges of RT proved useful in two ways. First, it enabled us to dissociate effects on accuracy from effects on RT. Second, we found that there is a subset of fast, inaccurate responses which are scarcely influenced by the drug. Removing these before data analysis increases the sensitivity of the visual learning task for detecting the effects of 8-OH-DPAT on accuracy. By implication, it is worthwhile to discourage rats from making such responses as far as possible. However, this increase in sensitivity may not generalize to other drug effects, particularly drugs that act to increase the proportion of short-latency responses.

Neither dose of 8-OH-DPAT abolished learning. The absence of drug × block interactions suggests a general depression of performance rather than strong interference with learning within problems.

Although the extended training between experiments 1a and 1b succeeded in cutting down the number of fast, uninformative choices and improving overall discrimination performance, we cannot say whether that was a consequence of the procedural changes, for example consistent use of moving stimuli and more trials per problem or simply a benefit of further practice. Whatever the reason, we can conclude that the drug effect was consistent over time, not confined to the rather lower levels of no-injection performance obtained in experiment 1a and not diminished by repeated exposure to the drug so far as that occurred in this study.

**Experiment 2**

As previously noted, there have been some claims of 'cognitive enhancement' by 5-HT1A antagonists. For example, Domeney et al. (1991) found that ondansetron caused marnosets to learn faster and choose more rapidly in a serial object discrimination reversal task. However, the negative effect of WAY-100579 in our rather demanding visual task, which bears some resemblance to serial reversal, in that reward associations change frequently, indicates that such enhancements are not universal.

Further, Domeney et al. (1991) reported a significant impairment in performance at the highest dose of 5-HT1A antagonist, so a bell-shaped dose–response relationship is also a possibility. It would then follow that doses of WAY-100579 lower than those used here should improve performance in our visual learning task.

**Discussion**

The discrimination impairment produced by 8-OH-DPAT was stable, appearing in all three experiments across 6 months of training and 10 exposures to the drug in low or high doses. This effect is consistent with predictions based on the pathological changes seen in AD (Bowen et al., 1994) and should be reversible by treatment with a 5-HT1A antagonist (cf. Stanhope et al., 1994a). Stanhope et al. (1994a, b, in press) discounted evidence for a small memory impairment after 0.1 and 0.3 mg/kg 8-OH-DPAT on the grounds that response latencies were increased and/or the rate of trial completion was slowed. Depression of such performance measures might indicate that the apparent memory impairment was due to non-specific effects. This conclusion does not necessarily follow since a slowing up of task performance might be a cause or consequence of some cognitive impairment (associated with excessive indecision). We argue that, in our procedure, it is possible to measure the effects of 8-OH-DPAT on discriminative accuracy, independent of either slowing or speeding up of motor responding.

In experiment 2, there was also evidence of impaired performance under 0.1 mg/kg of WAY-100579, but this effect has yet to be replicated. The result is a clear negative with respect to the possible cognitive enhancing properties of WAY-100579, at this dose in this apparatus. However, without more information on the dose-response for WAY-100579, which may resemble that for ondansetron in a similar task, this result should not be overinterpreted. Since experiment 2 provides preliminary evidence that rats are actually impaired in this task by 0.1 mg/kg WAY-100579 (cf. Domeney et al., 1991), in the discussion below we refer to generic drug impairments in performance, for convenience. Conclusions concerning the effects of WAY-100579 are provisional subject to the above reservations.

In all cases, the drug treatments produced some impairment but learning was still possible. These relative impairments cannot be ascribed simply to motor interference because the method of data analysis allowed the effects of a drug on discrimination accuracy to be dissociated from its effect on RT (see the analysis of results from experiment 1a). The possible contribution of other non-specific effects of the drug treatments are considered in turn below.

In experiments 1b and 2, there is some suggestion that saline injections caused suppression of performance in the first block of a problem, but accuracy nonetheless reached a high level by block 3. Therefore, the injection procedure alone may account for some of the impairment early in learning under the drug, but the sustained impairment in later blocks must be caused by the drugs themselves.

Serotonergic drugs have well-documented effects on appetite (Blundell, 1984; Cooper, 1989), but since these are opposite in sign for the compounds in use here, the pattern of results
obtained is not likely to find a ready explanation in terms of drug effects on motivation to respond for food reward. If our rats were hyperphagic after these relatively high doses of 8-OH-DPAT (Dourish, Kenet and Curzon, 1987; Dourish, Clark and Iversen, 1988), any increased motivation to respond might be expected to show itself as an increase in the proportion of short-latency (‘ill-considered’) responses. Figures 2(A) and 5(A) show that this was not the pattern of effects seen. And, as pointed out in the discussion of experiment 1a, the effects of 8-OH-DPAT on accuracy were actually clearer when short-latency responses were excluded from the analysis. Conversely, whilst the available evidence suggests that 5-HT₃ antagonists decrease the intake of (palatable) food (van der Hock and Cooper, 1994), there was no evidence that 0.1 mg/kg WAY-100579 increased the proportion of longer latency responses (Fig. 5A).

Because the rats were very experienced with the task and the specific constant stimulus to be learned about change unpredictably from one problem to the next, the impairments are unlikely to reflect state-dependent forgetting of rules or specific associations learned in previous sessions. With two to three problems per session and 10 sessions under each drug treatment (saline and 0.1 and 0.3 mg/kg 8-OH-DPAT) the rats had ample opportunity to learn in the drug state. The acquisition functions in Figs 1, 3 and 6 show that learning was possible in the drug states.

Possibly the drugs affected visual perception, rather than learning per se. This is unlikely in the case of 8-OH-DPAT because Carl et al. (1995) found that this treatment was without effect on visual discrimination at doses sufficient to cause impairment in spatial learning. In the case of 5-HT₃ antagonists, low-dose ondansetron improved performance in Domeny et al.’s (1991) visual task. However, the impairment seen at their higher dose might have been due to visual rather than cognitive disturbance. We have yet to determine whether 8-OH-DPAT and WAY-100579 impair simple discrimination learning with the visual stimuli used here and the parallel drop in the acquisition function seen in experiments 1a and (less clearly) 1b (Figs 1 and 3) is one possible consequence of visual disturbance. In Experiment 2 (Fig. 6), the pattern of effects was different (see below).

Acquisition to a lower asymptote is consistent with an effect on ‘performance’ which might be due to some kind of visual disturbance. In the present procedure, the use of wide-angle visual arrays to present both scene and (to a lesser extent) object stimuli (see Gaffan, 1991) and/or the movement required for good baseline performance, may contribute to task sensitivity to the effects of 8-OH-DPAT. Experiments with visual stimuli provided by (stationary) small-scale objects (Aggleton, 1985) may help to determine the psychological processes sensitive to drug treatments. Normal DA rats also make use of tactile information, when this is available (Huston and Aggleton, 1987). Determining the generality of drug impairments when additional cues are systematically introduced may also give useful information on the mechanism(s) of drug effects.

In experiment 2 (Fig. 6) there were indications that WAY-100576 and 0.3 mg/kg 8-OH-DPAT were particularly effective in later blocks. While the discriminability of the trial-unique stimuli from the constant one is not different on earlier and later trials, the potential for proactive interference among the trial-unique stimuli, which tend to share common elements, increases across trials. Successful performance requires that rats avoid more ‘familiar’ stimuli and interference among trial-unique stimuli might make this familiarity discrimination more difficult as trials proceed. This hypothesis can be tested by examining drug effects under conditions which further increase interference, e.g. extending problems for a further 10 trial block or deliberately manipulating stimulus characteristics to increase similarity or repetition among the positive stimuli.

Thus, although there are always non-specific effects to consider with systemic drug administration, the impairment in visual learning produced by the 5-HT₁A agonist 8-OH-DPAT confirms that cognitive effects can be demonstrated, in procedures allowing for effective dissociation of the motor effects of the drug (cf. Deacon, 1991; Everitt et al., 1994; Stanhope et al., 1994a,b; Stanhope, McLenachan and Dourish, in press). The previously reported improvement in delayed matching to position at 0.1 mg/kg 8-OH-DPAT (Cole, Jones and Turner, 1994) may be ascribed to differences between tasks and/or differences in the dose-response.

The results obtained with WAY-100579 suggest that 5-HT₃ antagonists warrant further investigation with these methods, at 0.1 mg/kg and lower doses. We do not rule out the possibility that WAY-100579 has cognitive enhancing properties at lower doses. The bell-shaped dose–response to ondansetron in object discrimination learning and its reversal (Domency et al., 1991) resembles that seen for the ‘attention-normalizing’ effects of haloperidol on latent inhibition (Dunn, Atwater and Kils, 1993), another paradigm with possible potential as a screen for cognitive enhancement (Weiner et al., 1992). Given that secondary (compensatory) effects are likely to engage when normal animals are given relatively high doses of drug, the beneficial effects of ‘cognitive enhancers’ may generally turn out to be confined to a narrow (low) dose range.

Acknowledgements
This work was supported by MRC grant G9812511N. H.J.C. was supported by Wellcome Trust Project Grant 026159. We thank Dr Helen Hodges for advice and Wyeth Research, UK, for the kind gift of WAY-100579.

Address for correspondence
H. J. Cassaday
Department of Psychology
University of Nottingham
University Park
Nottingham NG7 2RD
UK

References
A NOVEL TEST OF VISUAL LEARNING IN THE RAT


Gaffan E A, Woolmore A L (In press) Complex visual learning by rats. Learning and Motivation


