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Progress in Neuro-Psychopharmacology & Biological Psychiatry 27 (2003) 147–156
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

Intraventricular 5,7-dihydroxytryptamine lesions disrupt acquisition of working memory task rules but not performance once learned

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Accepted 18 November 2002

Abstract

The serotonergic system is implicated in learning and memory and its disorder, e.g. after 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') abuse. This study examined the effects of widespread depletion of serotonin (5-HT) using intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT) on the learning of a working memory task in the dark agouti (DA) rat.

The lesion impaired acquisition but not later performance of a nonspatial working memory rule, as measured using nonmatch to sample object recognition in the Y-maze. The lesion had a marginal effect on choice completion times over the course of testing. However, nonspecific effects did not provide a good account of the reduction in choice accuracy as this persisted when completion times were taken into account statistically. Similarly, in a second experiment, the same lesioned rats were slowed in the acquisition of spatial alternation in the T-maze. However, in the open field, there were no comparably long-lasting effects of the serotonergic depletion on line crossings and defecation, only a transient reduction in activity on the first day.

Together, these data suggest that the serotonergic system is important in the acquisition of working memory tasks in the rat and that this outcome was unlikely to be the result of nonspecific effects of the lesion.

Keywords: Learning; Rat; Serotonin depletion; Working memory

1. Introduction

The serotonergic system is implicated in learning and memory processes (Meneses and Hong, 1997; Gower, 1992) and their disorder, for example, the cognitive impairments associated with the recreational drug 3,4-methylenedioxymethamphetamine (MDMA). Whilst it is known that acute treatment with MDMA can result in cognitive deficits (Frederick and Paule, 1997; Morgan, 2000), there is little, if any, direct behavioural data on its longer-term effects in rats. Additionally although inconclusive, there is some evidence of serotonergic depletion in Alzheimer's disease (AD; Bowen et al., 1983, 1992; Reynolds et al., 1984) and in humans, AD results in working memory deficits when subjects must perform two tasks concurrently (Baddeley et al., 1991).

Many animal memory tasks are spatial (e.g. Warburton et al., 1997; Kant et al., 1998). As much of human learning and memory (and its dysfunction) are nonspatial in nature, then tests of experimental treatments on nonspatial memory tasks are of interest. Moreover, the contribution of the serotonergic system might itself vary depending on the spatial/nonspatial nature of the task (Carli et al., 1995).

There is evidence that the serotonergic system is necessary for nonspatial working memory processes from a study of the effects of (dietary) tryptophan depletion in normal human subjects (Park et al., 1994). By contrast, in animal studies, serotonergic depletion produced by *p*-chloroamphetamine (PCA) was without detectable effect on nonspatial working memory performance (Markowska and Wenk, 1991; Sakurai and Wenk, 1990). However, whilst treatment with PCA produced a persistent depletion of brain serotonin (5-HT), with systemic injection peripheral effects may confound interpretation of the results.

Abbreviations: AD, Alzheimer's disease; DNMS, delayed nonmatch to sample; 5,7-DHT, 5,7-dihydroxytryptamine; MDMA, 3,4-methylenedioxymethamphetamine; PCA, *p*-chloroamphetamine; 5-HT, serotonin.

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0278-5846/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0278-5846(02)00346-9

A nonspatial memory test was provided by Aggleton's (1985) continuous delayed nonmatch to sample (DNMS) procedure in the Y-maze. This is a recognition memory task that also taxes working memory. For present purposes, working memory is defined operationally by tasks that require the animal's response be based on some recently encountered stimulus that is relevant, i.e. the use of trial-specific information (Olton et al., 1979; Morris and Baddeley, 1988). The rats were not preoperatively trained because we were interested in effects on acquisition of the task as well as on retrieval.

The rat variant of the DNMS Y-maze task was based on those standard for monkey testing and has been found to be sensitive to the effects of scopolamine as a model for AD in both primates (Aigner and Mishkin, 1986) and rats (Huston and Aggleton, 1987). Boxes inserted at the ends of the arms of the maze contained a variety of objects and decoration and the rat was always rewarded for choosing the box that differed from the box chosen on the preceding trial. The next left/right position of the object box to be selected was irrelevant so the rat had to use nonspatial cues (provided by the features of the boxes) to complete the task. Because stimuli are not shown repeatedly within a session, this task does not rely on relative recency judgements within a single day's testing. In a second experiment, the rats were tested in a standard spatial working memory task, in which the correct response was to select the maze arm alternate to that encountered on the sample run. As well as relying on spatial processes, this task also requires memory for relative recency in order to distinguish the relevant sample run from previous sample and choice runs made that same day.

Intracerebroventricular 5,7-dihydroxytryptamine (5,7-DHT) was used to produce a widespread depletion of brain 5-HT (without the peripheral effects associated with systemic treatments). However, as 5-HT has widespread functions, it was possible that nonspecific effects of the lesion, such as motivational or motor effects, could confound interpretation of the results (Hole et al., 1976; Lorens et al., 1976; Kohler and Lorens, 1978). Disinhibited responding is a well-documented effect (of at least some methods) of serotonergic depletion (Soubrie, 1986) so the maze procedures were designed to reduce the number of choices made without proper inspection of the stimuli. In the Y-maze, rats were restrained at the choice point behind a transparent barrier through which the goalboxes were visible. Similarly, on the T-maze, low hurdles slowed down responding and provided an enclosed section within which the cues could be sampled before the final selection was made.

Because of the possibility of such nonspecific effects, we measured the time taken to complete each session of 10 trials in the Y-maze. Time taken per stimulus choice could not be accurately assessed, but the average time per choice within each session was analysed. Then, in Experiment 3, we used the open field to assess differences in activity and emotionality by measuring number of lines crossed and number of faecal boli excreted, respectively. Defecation has been widely used as a measure of anxiety, even in outbred strains (e.g. Sachdev and Saharov, 1998) and represents a validated measure of anxiety (Vanderwolf et al., 1988). The open field test was done last to allow 'blind' testing of rats run in the working memory tests.

2. Methods

All procedures were conducted under the United Kingdom Animals (Scientific Procedures) Act 1986 (Project Licence number PPL 40/1423).

2.1. Animals

Twenty experimentally naïve male dark agouti (DA) rats (Harlan-UK, Bicester, UK) weighing 205–240 g at the time of surgery were used. Throughout, rats were maintained on a 12:12-h light/dark cycle (lights on 08:00–20:00 h) and tested during the light phase, between 10 a.m. and 4 p.m. They were usually housed in pairs except during postoperative recovery when they were housed singly. Rats were fed a ration of chow (B&K Universal, Hull, UK) after testing each day, 7–10 g/rat, as necessary to motivate performance whilst maintaining weights at approximately 90% of free feeding weight. Water was freely available in the home cage. Weights were between 184 and 235 g at the beginning of the behavioural procedures and the rats continued to gain weight on this schedule.

2.2. Surgery

A total of 20 rats were allocated to two groups, for intracerebroventricular administration of 150 μg 5,7-DHT creatine sulphate (DHT, $n=12$: Sigma, Poole, UK) or the vehicle control injection (VEH, $n=8$) 10 μl 0.1% ascorbate artificial cerebrospinal fluid (aCSF: 60 mM NaCl, 10 mM $NaHCO_3$, 1.2 mM KCl, 250 mM NaH_2PO_4, 1.3 mM Na_2HPO_4, 250 mM Na_2SO_4, 500 μM $MgCl_2$ and 500 μM $CaCl_2$). Desipramine pretreatment (10 mg/kg ip; Sigma) was used to protect noradrenergic neurones (Bjorklund et al., 1975; Azmitia et al., 1978; Aspley et al., 1990). Like the vehicle for the neurotoxin, desipramine was also administered to control rats.

The rats were anaesthetised with halothane (4% in oxygen: Zeneca, Macclesfield, UK) and placed into a stereotaxic frame with atraumatic ear bars. Anaesthesia was maintained throughout surgery with 1–2% halothane. All injections were made bilaterally (5 μl/ventricle/min) at the following coordinates (mm from bregma): AP −0.8, ML ±1.5 and V −3.8 (mm from dura). The needle was then left in situ for a further 4 min after the infusion was completed. Coordinates were taken with the incisor bar set at 0 mm below the interaural line.

Immediately following surgery, all rats were given 2 ml of glucose/saline sc to aid recovery and prevent postsurgical

dehydration. They were also provided with additional bedding and palatable foods until food and water intake normalised and weights stabilised. After their initial postoperative reactivity subsided, rats were handled during this postoperative monitoring to prepare them for behavioural testing.

2.3. Tissue extraction and HPLC

At the end of Experiment 3 (a total of 17 weeks postoperatively), animals were stunned, cervically dislocated, then decapitated and the brains removed. The hippocampus, hypothalamus, frontal cortex and dorsal and ventral striatum were rapidly dissected over ice, placed individually in Eppendorf tubes, frozen on dry ice and stored at -80 °C until extraction.

Tissue samples were weighed frozen, then thawed and 1 ml of extraction solution (0.02% $Na_2S_2O_5$ and 0.1 M perchloric acid) was added. Samples were then probe sonicated (Soniprep 150 ultrasonic disintegrator, Fisher Scientific, Loughborough, UK), spun at 3500 rpm for 10 min and the supernatant stored at 80 °C. All samples for a particular region were extracted at the same time.

5-HT was separated and measured in the brain region extracts using HPLC with electrochemical detection (ECD) using a Hypersil C_{18} column (3 μm ODS; 10 cm × 4.6 mm internal diameter) with a mobile phase containing 0.15 M NaH_2PO_4, 1 mM EDTA, 0.5 mM 1-octanesulphonic acid and 14% methanol adjusted to pH 3.4 with phosphoric acid (1 M) and a flow rate of 0.3 ml/min. Tissue samples were extracted in antioxidant (1 ml 0.1 M perchloric acid containing 1.6 mM sodium metabisulphite), followed by sonication (30 s) on ice and centrifugation. Resultant supernatants were filtered (0.45-μm syringe filters, Gelman Sciences, Northampton, UK) and 5-HT quantified by ECD (Antec detector, Presearch, Hitchin, UK) using a potential of 0.65 V. Minimum level of detection was 20 fmol/20 μl injected onto the column.

2.4. Data analysis

All statistical analyses used a two-way mixed-design ANOVA with lesion as the between-groups factor (at two levels—DHT and VEH). For the neurochemical data, the dependent variable was 5-HT levels and brain region was the within-groups factor (at five levels for the five regions taken). All statistical tests used an alpha of .05. Further details of the behavioural analyses are given below.

2.5. Experiment 1: Y-maze

2.5.1. Apparatus

The animals were tested in a manually operated aluminium Y-maze. There were three converging identical arms, each 13 cm wide, 20 cm high and 15 cm long, leading to opaque guillotine metal doors at the entry to the goalbox. These doors could be raised by the experimenter on pulleys to allow the rat to see into and to enter or leave the goalboxes. The ends of each arm were open to permit insertion of the goalbox stimuli beyond the guillotine door into an area 20 cm long by 13 cm wide, creating a total arm length, including box length, of 35 cm. The guillotine door was used to shut the rat in with the selected goalbox. The arms each had a clear Perspex roof. All three arms of the maze were identical, and converged on a centre triangular space ($13 \times 13 \times 13$ cm) into which a trefoiled door made of wire mesh could be raised or lowered. This served the purpose of allowing the animal to look through from one arm to the other two and so visually inspect the boxes (when the guillotine doors were also raised). The guillotine door to the end of the arm just visited would be closed to prevent any access to or visual inspection of the immediately preceding goalbox. Lighting was supplied by normal overhead fluorescent lighting of the same brightness as the holding room. In addition, each arm of the maze was illuminated by three 2.8-W bulbs mounted in the Perspex roof (on the box side of the guillotine metal doors) to ensure that the boxes were well lit and encourage visual inspection of the stimuli that they contained. Immediately above the goalbox position were two small holes in the Perspex roof through which the reward of two 45-mg Campden reward pellets (Campden Instruments, Loughborough, UK) was dropped as dictated by the experimental schedule.

2.5.2. Stimuli

Twenty-two pairs of plywood boxes served as stimuli and fitted into the ends of the maze arms. They each had a 17-cm long by 12-cm wide base and were 15 cm high at the back. At the top of the back surface, a roof formed an enclosed section of the box that was 8.0 cm deep. The front and remaining top face of the box were left open, but the top would be covered by the Perspex lid of the maze and the front face by the metal door when the box was in situ. Each pair was identical, but made distinctive from every other pair by using different configurations of small plastic toys or household items such as matchboxes or plastic cups. The walls of each pair also varied in colour and pattern to give overall brightness and contrast differences. The use of matched pairs of boxes meant that rats could be tested for recognition of a sample box without odour cues.

2.5.3. Pretraining and introductory trials

Pretraining began 3 weeks after surgery. First rats were given reward pellets in the home cage (over 3 days) and further handling. This was followed by 7 days of habituation to the apparatus: Rats were simply placed in the maze, initially in pairs and later singly, with food pellets distributed throughout. On later days, the arms and doors of the maze were moved to accustom the rats to the operation of the maze and the location of the food pellets was restricted to the arm ends. Then followed 12 days of ‘introductory trials’ in which each rat was individually given two trials of

DNMS using the planned experimental procedure, but with shaping as necessary (e.g. dropping reward pellets into boxes to encourage full goalbox entry). Thus, this phase familiarised the rats with the apparatus and procedure and allowed us to eliminate those that could not be tested.

2.5.4. Experimental procedure

The experimental trials began 7 weeks postoperatively. There were 32 sessions of testing, with 10 trials for each rat per session. Each session therefore used 11 pairs of boxes out of the 22 pairs, 1 as the first sample and 10 to provide the subsequent choices. The sequences of 11 boxes used and their positioning to the left or right of the start box were semirandomly generated by computer and were the same for every rat on a given day. Each left/right direction was selected an equal number of times and never used more than twice consecutively. The DNMS procedure was as follows: one of the first pair of identical boxes in the list was placed in the arm of the maze to be used as the start box. The corresponding matched box of the pair was then placed in either the arm to the left or right of the start box. One of the second pairs of boxes was then placed in the remaining arm. The correct choice was always a member of the next designated pair of boxes, avoiding the box identical to the last-visited sample.

A single trial began with the rat placed into an arm of the maze between the central mesh barrier and the closed door to the first sample (or start) box. The door to the sample was then raised and when it entered, the rat was rewarded with two food pellets dropped through the Perspex roof. The door was lowered in order to enclose the rat in the start box for approximately 2–3 s (to find and eat the pellets) after which the door was raised and the rat was allowed to leave the start box and reenter the maze arm. The door was then closed behind the rat and after 3 s, the doors leading to the other two boxes were raised so that from the central mesh, the rat could view both. One was always identical to the start box and the other was always different. After 3 s to observe the boxes, the central mesh was raised. If the animal entered the correct box, i.e. the one that differed from the sample, it was enclosed by lowering the box door for 2–3 s and rewarded with two pellets. If it chose the wrong box, the rat was still shut in for the same 2–3 s but not rewarded. The choice criterion was a full entry, scored when all four feet crossed the entrance; in practice, this was when the rat could be shut into the box. Following an error, the same two boxes were used again but switched in position to avoid the development of side preferences. Correction runs were continued until the rat chose the correct box, up to a maximum of three attempts; on the fourth run within the trial, only the door to the correct choice would be opened, effectively forcing the rat to enter that box where it was rewarded as usual.

The last-entered box then became the sample for the next trial. The boxes in the other arms were replaced (whilst the rat remained confined in the box last selected) with the duplicate of this new sample and the next box designated on the data sheet. This latter box provided the new correct 'nonmatch to sample' and the box identical to the one the rat occupied would be incorrect. Thus, the procedure was continuous with the last entered box becoming the next trial's sample until all 10 trials were completed. The rat was then returned to the home cage.

We took an overall measure of response time for each rat. This was the total time (in seconds) taken to complete 10 trials, from entering the first sample box to enclosure in the final box and so included any repeat correction runs. Latencies could not be recorded to an acceptable level of accuracy on a choice-by-choice basis, so the completion times reflect all the delays introduced by differences in the rats' rate of movement.

The completion time measure was introduced on Day 3, providing a total of 30 days of data. Testing was shared among six experimenters who are blind to the lesion allocations, and throughout each experiment, rats were run 4–5 days/week.

2.5.5. Statistics

For the accuracy data, the dependent variable was percentage correct. For the completion data, the dependent variable was the mean time taken per choice (whether correct or incorrect) within a session. These latency data were subjected to a square root transformation to render it suitable for parametric analysis. Lesion (DHT vs. VEH) was the between-subjects factor and blocks (five lots of 6 days) was the within-subjects factor for both accuracy and completion time data. Post hoc comparisons were by *t* test (two-tailed).

We also ran the accuracy analysis with mean choice completion time per choice as covariate to see how far group differences in accuracy were likely to be related to nonspecific effects on performance.

2.6. Experiment 2: T-maze

2.6.1. Apparatus

The T-maze was constructed from plywood. The start arm was 80 cm long and each of the choice arms was 60 cm long. All the arms were 10 cm wide. At the junction of the sample and choice arms was a square space 10×10 cm. Hurdles 3 cm high, made of wooden dowelling (1 cm diameter), were positioned at the point of entry to the choice arm and 30 cm further along the choice arms. There were food wells at the ends of the arms, 4 cm in diameter, positioned 5 cm in from the end of the choice arms. Two wooden blocks were used to enclose the rats on the arms. The outside edges were trimmed with a wooden lip, 1 cm high throughout, except at the very ends of the choice arms where it was 6.5 cm high. The maze was elevated on 30-cm stilts from a table in a brightly lit room.

2.6.2. Experimental procedure

The experiment began 2 weeks after the end of Experiment 1. Rats were first accustomed to the maze over 5 days

until they ate and explored freely. Then followed 16 days of spatial alternation. Rats were placed on the start arm. Sampling of the designated left/right arm was 'forced' because the wooden block prevented access to the alternate arm. The rat was confined on the sample arm for the time required to find and eat two reward pellets placed in the food well (on average 2 s). It was then returned to the start arm and blocked in. After a 3-s delay, the rat was released to make its selection. The choice criterion was that the rat should clear the second hurdle. Between the hurdles, rats were allowed to correct their choices. If the correct arm was selected (i.e. the arm opposite to that of the forced entry), the rat was enclosed with the block and given two reward pellets. If the wrong arm was selected, the rat was enclosed without reward for approximately the same time it would normally take to eat two reward pellets (i.e. 2 s). There were six trial pairs (of one forced then one free choice) per day, run without any within-trial correction. A semirandom sequence of left and right was used for the rewarded sample runs, with the constraint that there were no more than two consecutive runs in the same direction within a single session. These were the same for each rat on any day.

2.6.3. Statistics

There were four blocks of 4 days (to assess the effect of the lesion on a more rapid acquisition over fewer days of testing), again in a Lesion × Blocks mixed design.

2.7. Experiment 3: Open field

2.7.1. Apparatus

The experiment was conducted in a standard open field apparatus. This consisted of a white Formica, circular base with a diameter of 84 cm, enclosed by a surrounding wall 32 cm high, also of white Formica. The floor was scored with lines in the form of two concentric circles (inner circle diameter 19 cm, outer circle diameter 51 cm), intersected by straight lines radiating out from the centre so that the floor was divided into 24 sections. The field was brightly illuminated, by the normal fluorescent lighting of the room, so that there were no shadows.

2.7.2. Testing procedure

This began immediately after the end of T-maze testing. Activity and emotionality measures were taken over three consecutive days. Each rat was placed individually in the centre of the open field and observed for 10 min. This duration corresponds with the average time any rat took to complete the maze testing sessions. The number of lines crossed and faecal boli excreted were checked off on a score sheet.

2.7.3. Statistics

Here the within-subjects factor was days, of which there were three. There were two dependent variables, activity, measured as number of lines crossed, and emotionality, measured as number of faecal boli excreted within a session.

3. Results

One rat died during surgery. Seven rats (two VEH and five DHT) were dropped before the experiment began and were excluded from the neurochemical analysis, because they did not move about freely in pretests on the maze. One rat (DHT) was later dropped for consistently refusing to run. This left 11 rats in the final behavioural analyses (six VEH and five lesioned with 5,7-DHT).

3.1. Neurochemistry

The means and standard errors for the 5-HT levels in nanomoles per milligram for DHT and VEH groups are presented in Table 1. From the means, it is clear that 5-HT was depleted in the DHT relative to the VEH group in every region, as would be expected.

Statistically, there was a significant main effect of lesion [$F(1,9) = 9.08$, $P < .05$], which arose because the DHT group had overall less 5-HT than the VEH group. There was also a significant main effect of region [$F(4,36) = 7.91$, $P < .001$] because as expected there were different levels of 5-HT in the different brain regions sampled. However, the interaction was nonsignificant [$F(4,36) = 1.65$, $P > .05$] so the lesion was effective across all the brain regions sampled.

3.2. Experiment 1: Y-maze

3.2.1. Completion times

Fig. 1 shows that the DHT group initially took longer to complete their choices but that they got faster over the course of testing. Statistically, there was a significant main effect of blocks [$F(4,36) = 6.62$, $P < .001$], but there was no significant main effect of lesion [$F(1,9) = 2.79$, $P > .05$], so there was no overall difference between the DHT and VEH

Table 1
Effects of 5,7-DHT on 5-HT in different brain regions, 17 weeks postadministration

Brain region	Group	Mean	S.E.M.	% Loss
Hypothalamus	DHT	100.4	27.9	48
	VEH	193.1	41.7	
Hippocampus	DHT	38.5	21.4	44
	VEH	69.1	48.9	
Frontal cortex	DHT	323.7	193.4	54
	VEH	711.1	132.2	
Ventral striatum	DHT	172.4	34.4	20
	VEH	215.3	67.8	
Dorsal striatum	DHT	149.29	14.4	32
	VEH	219.6	47.7	

DHT: $n = 5$; VEH: $n = 6$.
All data are given as nanomoles per milligram. n = available sample size; S.E.M. = standard error of the mean.

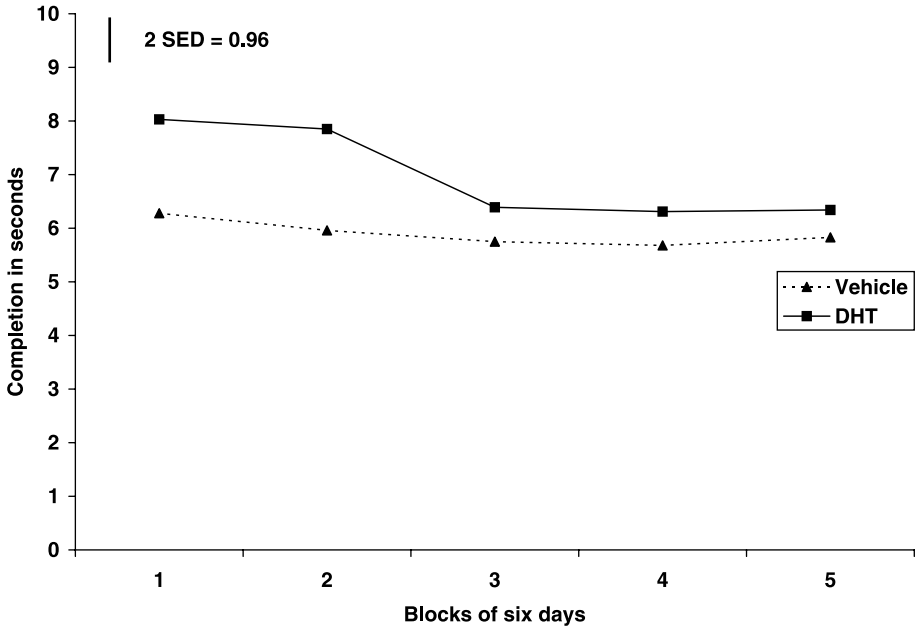

Fig. 1. Effects of 5,7-DHT lesion on mean time to complete each stimulus choice of DNMS in Experiment 1. Completion time is shown in seconds after square root transformation. Bar at top left (2 S.E.D.) shows two standard errors of the difference of the mean.

groups. The Lesion × Blocks interaction approached significance [$F(4,36) = 2.54$, $P = .057$]. Because completion time changes could confound interpretation of the accuracy data, we followed up on the marginal interaction with *t* tests. However, although Fig. 1 shows the difference between lesion and VEH groups to be greater in the early blocks, there was no particular block on which the lesioned group was significantly slower [maximum $t(9) = 1.87$].

3.2.2. Accuracy scores

The first 2 days accuracy data for which the corresponding completion times were not available were dropped to allow the data to be presented in parallel. This means that the blocks of days shown in Figs. 1 and 2 and analyses applied to percentage correct and latency scores are directly comparable.

Both DHT and VEH groups were performing at above chance level from the first block of trials reflecting rats' natural preference for novel stimuli. The groups then clearly diverged with the VEH outperforming the DHT rats. Later in testing, the groups came together because the DHT group improved, and this convergence was sustained over further blocks of testing.

Statistically, there was a significant main effect of lesion [$F(1,9) = 5.48$, $P < .05$], reflecting the fact that the DHT rats performed worse on DNMS than the VEH group. There was also a significant main effect of blocks [$F(4,36) = 5.15$, $P < .01$], showing that overall DNMS accuracy fluctuated

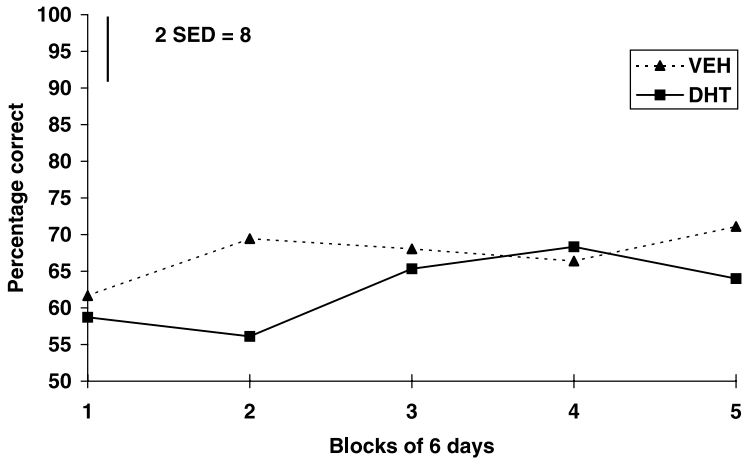

Fig. 2. Effects of 5,7-DHT lesion on accuracy in a DNMS task in Experiment 1. Bar at top left (2 S.E.D.) shows two standard errors of the difference of the mean.

over time. The significant interaction between lesion and blocks [$F(4,36) = 3.93$, $P < .01$], confirms that there was variation in the size of the lesion effect on accuracy over the course of acquisition. By t test, the impairment in the DHT group relative to VEH was significant only at Block 2 [$t(9) = 4.27$, $P < .01$], but not at other points in the testing [maximum $t(9) = 1.77$]. This pattern of effects suggests that the lesion delayed acquisition of the DNMS task, relative to controls.

Analysis of covariance showed that the Lesion × Blocks interaction remained significant when the corresponding completion time scores were used as covariates [$F(4,35) = 6.22$, $P < .01$].

3.3. Experiment 2: T-maze

The rats rapidly learned to alternate with some apparent early impairment in the DHT group (Fig. 3).

There was a main effect of blocks [$F(3,27) = 8.17$, $P < .001$]. However, there was no overall effect of lesion [$F(1,9) = 2.10$]. Whilst the Lesion × Blocks interaction was also nonsignificant [$F(3,27) = 1.84$], trend analysis showed that it was significant in the quadratic [$F(1,9) = 6.72$, $P < .05$]. This confirms that accuracy changed differently in VEH and DHT groups over the course of testing, as illustrated in Fig. 3. Post hoc comparisons by t test showed that the DHT performed worse that the VEH rats on Block 1 [$t(9) = 3.02$, $P < .05$], but not subsequently [t's(9) < 1.5].

3.4. Experiment 3: Open field

The summary statistics for number of lines crossed and number of faecal boli in the open field test are presented in Table 2.

The means suggest that the DHT rats were less active than the VEH controls on each of the 3 days. The number of faecal boli was very similar in both groups. However, contrary to expectation, there was no overall significant effect of lesion for line crossings [$F(1,9) = 3.6$, $P = .09$]. There was a marginal effect of days [$F(2,18) = 3.14$, $P = .07$], but the Lesion × Days interaction was not significant [$F(2,18) < 1$]. Since this negative result is important to the argument that nonspecific effects provide an unlikely account of the observed working memory deficits, we also carried out unplanned comparisons. These showed that the marginal effect on activity was confined to the first day of testing [$t(9) = 3.23$, $P < .01$], but subsequently it was nonsignificant [t's(9) < 1.5]. For faecal boli, again there was a marginal effect of days [$F(2,18) = 3.04$, $P = .08$], but there was no sign that the lesion had any effect on the number excreted either as a main effect or in interaction with days (both F's < 1). This lack of effect was confirmed by the post hoc comparisons [maximum $t(9) = 0.1$].

Table 2
Effects of 5,7-DHT lesions on measures of line crossings and faecal boli in the open field

	Day 1	Day 2	Day 3
(A) Mean ± S.E.M. number of line crossings			
VEH	129.33 ± 10.29	140.50 ± 11.60	121.50 ± 14.00
DHT	83.80 ± 9.23	118.40 ± 25.40	89.40 ± 17.86
(B) Mean ± S.E.M. number of faecal boli			
VEH	1.83 ± 0.83	3.50 ± 0.81	2.33 ± 0.92
DHT	2.40 ± 0.6	3.40 ± 0.93	2.20 ± 0.97

4. Discussion

The lesion depleted 5-HT levels relative to the VEH group consistently across the various brain regions sampled.

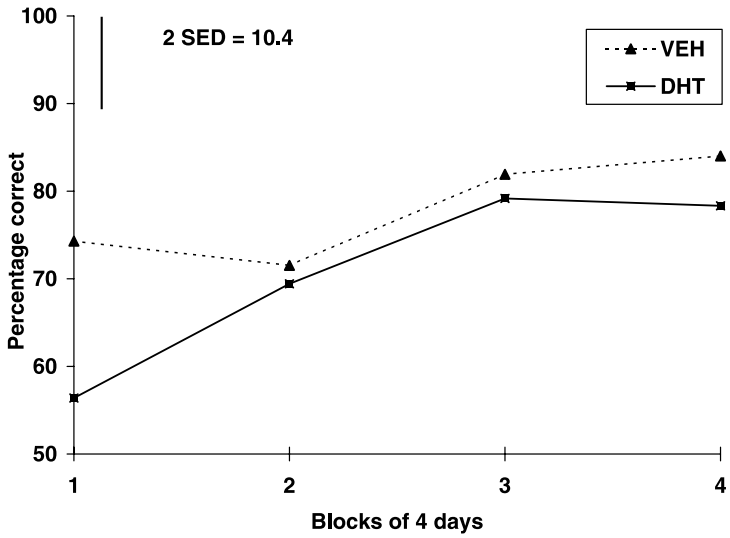

Fig. 3. Effects of 5,7-DHT lesion on spatial alternation in Experiment 2. Bar at top left (2 S.E.D.) shows two standard errors of the difference of the mean.

It is likely that some recovery due to sprouting will have occurred by 17 weeks (Baumgarten et al., 1974; Azmitia et al., 1978), in which case, the assay underestimates the size of the initial depletion. Moreover, the fact that we saw some impairment with a relatively modest depletion would seem to underscore the role of the serotonergic system in the normal acquisition of working memory rules (at least under our conditions of testing). We predict that the use of a higher dose of 5,7-DHT would result in bigger behavioural effects, though with a likely increase also in adverse effects (Snape et al., 1994). However, reliable decreases in extracellular 5-HT are typically seen with relatively substantial depletions (greater than 60%), so the behavioural effects observed here could instead be the result of compensatory changes in spared neurones, in which case the behavioural effect of a larger depletion would also be different (Hall et al., 1999).

The level of serotonergic depletion seen here is similar to that which can be produced with MDMA (Colado et al., 1993; O'Shea et al., 1998). The mechanisms of MDMA-induced neurotoxicity are different from those of 5,7-DHT (Snape et al., 1994). However, since the spread and size of the serotonergic depletion produced by treatment with MDMA in DA rats is comparable to that seen here, our results suggest that (in consequence of its longer term neurotoxic effects) MDMA may similarly delay the normal acquisition of working memory rules.

The DA strain was appropriate because of their good visual abilities, necessary for the Y-maze task. Relatively high emotionality (Mechan et al., 2002) may have contributed to the number of exclusions but these were appropriate since nonspecific effects on task performance were not of interest. Moreover, the rats excluded because they did not complete the maze pretraining were predominantly from DHT lesion group (six of the nine exclusions, plus one that stopped running at a later stage). This suggests that the failure to perform was a result of nonspecific effects of the lesion.

In principle, this number of exclusions might have been further reduced if preoperative maze training had been employed. However, it would not then have been possible to test for lesion effects on acquisition of the working memory rules. As the results turned out, the effects of serotonergic depletion were confined to learning the rules as distinct from their later performance.

In addition, it is possible that the widespread depletion of 5-HT here may have resulted in compensatory increases in other monoamines that could mediate nonspecific effects. It would therefore be useful in future studies to measure the levels of noradrenalin and dopamine in order for any such changes to be taken into account.

4.1. Experiment 1: Y-maze

Both lesioned and nonlesioned groups learned the DNMS rule, but serotonergic depletion reduced performance accuracy in the early stages of acquisition. This recognition memory impairment was significant only in the second week of testing as the control group first moved above chance-level performance. All training was postoperative, so this restricted effect of the lesion supports the hypothesis that the serotonergic system contributes to nonspatial learning. Once the lesioned group acquired the task (by the third week of testing), they were able to perform DNMS, so there was no evidence that the lesion also produced retrieval or performance deficits (that we would expect to be sustained for the duration of testing).

Slowed response times could be a cause of cognitive impairment, in which case the lesion effects on accuracy seen here would be secondary. For example, it is possible that rats responding more slowly had to remember the sample longer and that their decrease in accuracy therefore reflects the greater mnemonic demands of an effective increase in the retention interval. Thus, slowed response times could reflect nonspecific effects that confound interpretation of accuracy data.

The most likely nonspecific effect that could confound choice accuracy after serotonergic depletion is behavioural disinhibition (Harrison et al., 1999). However, our lesioned rats took more rather than less time to complete at the stage of testing when their choices were inaccurate. In an automated task variant, we have previously found that there is a positive association between choice accuracy and response time in untreated rats, and that treatments that may hasten or slow responding can have dissociable effects on accuracy (Cassaday and Gaffan, 1996). Behavioural disinhibition is therefore an unlikely account of the observed effects on accuracy.

The effects of serotonergic treatments on food intake are well documented (see e.g. Cooper, 1992), but whatever the direction of any effects of our lesion on appetite, these would result in response time differences. For example, increased motivation to respond for food might be associated with rapid responses made without proper inspection of the stimuli and so reduced choice accuracy; decreased motivation to respond for food might reduce impulsive responding and so improve choice accuracy.

However, there is no evidence for the possibility that nonspecific effects mediated the lesion-induced impairment in accuracy because this persisted when completion time was taken into account statistically.

The lack of a long-lasting effect of the lesion could mean that the nonspatial working memory task was insufficiently taxing. However, this seems an unlikely explanation of the present results, as the control group accuracy remained relatively low. This baseline and the tendency for the lesion to increase completion time together made investigation of delay-dependent deficits impracticable. With a differently mediated effect, scopolamine treatment as a model for AD impaired performance in the same task even at the shortest retention interval consistent with its implementation (Huston and Aggleton, 1987).

4.2. Experiment 2: T-maze

The 5,7-DHT lesion produced a transient impairment in spatial alternation but this too was not sustained and all the rats soon reached a high level of performance. Thus, there was no indication that the increased requirement to judge relative recency added to the observed behavioural deficits. Rats learn spatial alternation very readily and this may account for the fact that the groups' accuracy soon converged.

Such a short-lived effect of serotonergic depletion apparently contrasts with the more persistent effect (seen over a longer period of testing) in Experiment 1 and could reflect neuropsychological dissociation of the systems necessary for spatial and nonspatial memory (Carli et al., 1995). Alternatively, at this postoperative interval, the more rapid behavioural recovery could also be a consequence of more advanced functional recovery due to sprouting (as discussed above). Rats' tendency to spontaneously adopt spatial strategies is the major obstacle to their learning nonspatial DNMS so we had to give the rats the harder nonspatial task first. Thus, we may have underestimated the 'real' spatial impairment associated with this kind of lesion because of the necessary order of experiments. A further possibility is that the lesioned rats showed impaired 'flexibility' in switching from one set of rules (DNMS) to another (spatial alternation). However, the fact that the lesion effect again depended on the stage of acquisition in this (easier) task suggests that it was more likely to reflect the fact that serotonergic depletion similarly affected the learning but not the later retrieval of the working memory rule or performance of the task.

Whilst the Y-maze used in Experiment 1 was enclosed so that rats would not be distracted from the objects of interest by the extra-maze cues, the T-maze of Experiment 2 was open, for precisely the opposite reason. In the absence of intramaze cues, rats must navigate by the use of extramaze cues to perform spatial alternation. Whilst the use of an open elevated maze in a brightly lit room may have introduced anxiety as a nonspecific effect (Silveira et al., 1993), the results of Experiment 3 argue against the interpretation of performance deficits in terms of effects on emotionality.

4.3. Experiment 3: Open field

Before the start of experimental testing, we had already excluded rats whose reactivity made them unsuitable for testing on the working memory tasks. This final test was intended to determine whether the performance impairment of the final sample finds any obvious account in terms of enduring (nonspecific) effects. This was not a fine-grained analysis of activity. We did not distinguish movement in the inner and outer annuli and we used a 10-min sample of activity as this corresponded with the average time to complete the Y-maze task (in neither task were the data broken down into smaller time bins).

The open field presents a novel environment, usually sensitive to the effects of serotonergic depletion, though the direction of behavioural effects seems to depend on the methods of (nonspecific) depletion in use (Hole et al., 1976; Lorens et al., 1976; Kohler and Lorens, 1978). However, selective 5,7-DHT lesions also seem to have inconsistent effects, either increasing (Vanderwolf, 1989) or decreasing activity (File and Deakin, 1980). In the present study, activity was reduced in the lesioned groups, but this effect of the lesion was temporary, confined to the first day of testing. Thus, there was no evidence that effects on activity or emotionality provide a likely account of the working memory deficits seen in Experiments 1 and 2.

The completion time and open-field data together suggest that these cognitive effects of the 5,7-DHT lesion were not secondary to nonspecific effects on emotionality or exploration.

5. Conclusion

We conclude that the serotonergic system contributes to the acquisition but not the performance of a nonspatial working memory task, as measured by DNMS choice accuracy. This behavioural effect was relatively transient but nonetheless statistically reliable. We also saw evidence of a temporary impairment in T-maze alternation, consistent with some serotonergic contribution to the acquisition of a spatial working memory rule also. It is unlikely that the impairment seen is a result of nonspecific effects of serotonergic depletion.

These findings suggest that serotonergic depletion contributes to the difficulty in learning new tasks characteristic of AD and that similar cognitive impairment may result from the effects on the serotonergic system of long term use of MDMA.

Acknowledgements

This work was supported by MRC grant G9626839. We thank Dennis Vardy for building the mazes and Sarah Orchard and Teresa Sharp for helping with the behavioural testing.

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