THE EFFECT OF NONSPATIAL WATER MAZE PRETRAINING IN RATS
SUBJECTED TO SEROTONIN DEPLETION AND MUSCARINIC
RECEPTOR ANTAGONISM: A DETAILED BEHAVIOURAL
ASSESSMENT OF SPATIAL PERFORMANCE

by

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Abstract

A detailed behavioural analysis of water maze spatial performance in the rat was utilized to determine the effect of single and combined administration of p-chlorophenylalanine (PCPA: 1000mg/kg, i.p.), an inhibitor of serotonin biosynthesis, and scopolamine hydrobromide (SCO: 1.0mg/kg, i.p.), a muscarinic receptor antagonist. In some groups a water maze pretraining regimen known as non-spatial pretraining (NSP) was used to familiarize the animals with the general requirements of the task before spatial training was begun. In addition to the water maze task the rats were also assessed for motoric performance on a beam walking test. The results showed that: a) depletion of serotonin with PCPA had no effect on water maze performance, b) antagonism of muscarinic receptors produced impairments in spatial and sensorimotor function in naive rats but neither effect was observed in rats first given NSP, c) combined disruption of muscarinic and serotonergic function produced a severe deficit in spatial performance that was only partially alleviated by NSP, d) there was an association between poor maze acquisition scores and a high incidence of sensorimotor dysfunction, and e) antagonism of muscarinic and serotonergic function alone and in combination produced impairments in motoric function. The role of cholinergic and serotonergic systems in learning and memory is discussed.

Key words: Learning, Memory, Behaviour, Spatial Navigation, Hippocampus, Neocortex, Acetylcholine, p-Chlorophenylalanine, Scopolamine, Beam Task.

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TABLE OF CONTENTS

CERTIFICATE OF EXAMINATION / ii

ABSTRACT / iii

ACKNOWLEDGEMENTS / iv

TABLE OF CONTENTS / v-vi

LIST OF FIGURES / vii

LIST OF TABLES / viii

LIST APPENDICES / ix

CHAPTER 1 INTRODUCTION / 1

1.0 Introduction

CHAPTER 2 MATERIALS AND METHOD / 3

2.1 Subjects / 3

Drug Administration and Protocol / 5

Testing Materials / 5

2.4 Behavioural Testing Procedure / 6

2.5 Behavioural Measures / 8

2.6 Statistical Analyses / 9

CHAPTER 3 RESULTS / 9

3.1 Water Maze / 9

3.2 Balance Beam / 16

3.3 Correlations / 19

CHAPTER 4 DISCUSSION 21

4.0 Discussion / 21

APPENDIX 1 POOL DIVISION FIGURE / 28

REFERENCES / 29

VITA / 35

LIST OF FIGURES

Figure	Description	Page
1	Hidden and visible platform search time, direct swims, and probe trial swim time.....	13
2	Distribution of swim time, percent of platform contacts that were deflections and swimovers, and loops and passes.....	14
3	Beam task traverse time and slips and falls	18

LIST OF TABLES

Table	Description	Page
1	Group designation and drug administration protocol.....	4
2	Correlation between water maze hidden platform measures.....	20

LIST OF APPENDICES

Appendix	Page
Appendix I Pool Division Figure	29

1. Introduction

Recent investigations in behavioural neuroscience have made progress in the field of spatial learning and memory. In particular, the development of novel training techniques and the adoption of detailed behavioural analyses have led to an increased understanding and re-examination of some of the existing work in the field [1,28,30,46]. The learning paradigm of choice in these studies has been the water maze [20,21], a task in which rodents are placed in a pool of water and required to search for a submerged platform whose position can be readily determined from information acquired from distal cues [21,33]. This task, although seemingly simple, is quite complex because it requires that the animal learn, in addition to the spatial location of the platform, various nonspatial components such as how to swim, where to swim (i.e. away from the wall), how to mount the platform, and that the platform serves as the only refuge from the water [5,44]. Thus, as the water maze is typically used, unambiguous assessments of spatial learning can be confounded by the various nonspatial components of the task.

To deal with this problem, Morris [22] developed a pretraining procedure that was designed to familiarize the animal with the nonspatial components of the task, while withholding spatial information. During nonspatial pretraining (NSP), the animal was given a series of training trials in which the location of the submerged platform was varied from trial to trial and a thick black curtain was hung around the circumference of the pool to prevent the animal from seeing distal cues. The elimination of distal cues and the random location of the platform allowed the animal to become familiar with the general requirements of the task while withholding spatial information. By combining NSP with

detailed behavioural analyses, inferences of learning, based on the rat's behaviour, became less confounded by the various nonspatial components of the task. This approach has been particularly useful when inferring learning in animals that have received various forms of experimental manipulations. For example, animals that were given N-methyl-D-aspartate (NMDA) or muscarinic receptor antagonists had been reported to display poor spatial performance [12,14,22,35,40,44]. However, if the animals were first given NSP, their performance scores were indistinguishable from controls, suggesting that neither of these receptor populations were necessary for spatial learning [1,6,16,28,29]. Detailed examinations of the performance of NSP rats vs. naive rats have revealed that the naive animals exhibited various sensorimotor disturbances associated with the nonspatial components of the task that likely interfered with their ability to acquire the necessary spatial information needed to successfully complete the task. Thus, NSP and detailed analyses of behaviour have clarified the role of some neurotransmitter receptor populations in spatial learning and memory.

In this thesis, we sought to examine the effect of separate and combined antagonism of muscarinic and serotonergic systems on spatial navigation in the rat. This particular form of combined antagonism is particularly interesting for several reasons. First, it is known that the cholinergic cells of the basal forebrain and the serotonergic cells of the brainstem provide a form of ascending control over the electrical activity of the hippocampus and neocortex [38,39]. This finding is relevant because several studies have suggested that the integrity of these structures is important for the performance of spatial tasks in naive rats [18,19,23,34,47]. Secondly, this preparation reproduces some of the behavioural and

neurotransmitter correlates observed in patients with Alzheimer's disease [2,8,50], and thus may be a useful animal model in studying the cognitive impairments observed in these patients [10].

Previous research has shown that antagonism of serotonergic function alone does not prevent acquisition of the water maze task in naive rats. However, when serotonergic antagonism is combined with muscarinic antagonism naive rats display profound deficits in water maze acquisition [24,26,27,37]. However, since none of these studies utilized NSP and a detailed behavioural analysis, it is not known whether the disruption of performance was due to an inability to learn the location of the platform, or an inability to use the appropriate behaviours and strategies necessary to successfully navigate to the platform. Therefore, in this paper we sought to examine the effect of this form of antagonism in rats made familiar with the nonspatial components of the task and to use a detailed behavioural analysis to document the occurrence of possible sensorimotor dysfunction during testing.

2. Materials and Methods

2.1 Subjects

The subjects were 96 male hooded Long-Evans rats weighing 300-500g. They were housed in single hanging cages and maintained on a 12:12 hr light/dark cycle. In addition to receiving ad libitum access to food and water, rats treated with p-chlorophenylalanine (PCPA) received sucrose sweetened wet mash (post-treatment) to reduce the weight loss associated with serotonin depletion [11]. On several occasions prior to behavioural testing the rats were removed from their home cages and individually handled. Handling consisted of placing several rats on a raised cart in the laboratory for at least three sessions

TABLE 1.
Group designation and drug administration protocol

Group	(n)	Day								
		1	2	3	4	5	6	7	8	9
NSP PCPA+SCO	10	NSP	NSP	NSP	NSP	PCPA	PCPA	-	-	SCO+Train
NSP PCPA	8	NSP	NSP	NSP	NSP	PCPA	PCPA	-	-	Train
NSP SCO	12	NSP	NSP	NSP	NSP	-	-	-	-	SCO+Train
Naive PCPA+SCO	12	-	-	-	-	PCPA	PCPA	-	-	SCO+Train
Naive PCPA	10	-	-	-	-	PCPA	PCPA	-	-	Train
Naive SCO	12	-	-	-	-	-	-	-	-	SCO+Train
Gum Arabic	6	-	-	-	-	G.A.	G.A.	-	-	SAL+Train
Saline	6	-	-	-	-	-	-	-	-	SAL+Train

NSP=Nonspatial Pretraining; G.A.=Gum Arabic; Sal=Saline; SCO=Scopolamine

PCPA=p-chlorophenylalanine; Train=Begin Water Maze Training.

prior to water maze training. All behavioural testing occurred between 0900 and 1700.

2.2 Drug administration and protocol

The drug administration protocol and group assignments are outlined in Table 1. PCPA (1000mg/kg) was suspended in a solution of gum arabic (0.5%) in saline (0.9%) and administered in single (500mg/kg, i.p) injections dispensed on each of two consecutive days. This dose and administration protocol was chosen because it has been shown to reduce whole brain levels of serotonin by 90.6% and 5-hydroxyindoleacetic acid by 91.8% [11]. This dose of PCPA also produces slight decreases in dopamine (14%) and norepinephrine (23%) levels [11]. Scopolamine hydrobromide (SCO) was administered in a single (1.0 mg/kg, i.p) injection. This dose was chosen because it has been shown to impair spatial performance in experimentally naive rats but not in NSP rats [16,29] and to produce a marked impairment in the electrophysiological activation of the neocortex [38]. A peripherally acting cholinergic antagonist was not used in the present study because previous research has shown that it does not produce a behavioural impairment in the water maze [37,48]. Water maze spatial training began three days after the final injection of PCPA and 20 minutes after the injection of SCO.

2.3 Testing materials

2.3.1 Water maze

The water maze was a circular pool (1.5 m in diameter and 45 cm deep) with walls and floor painted white. The maze was located in a room with various distal cues that could be occluded by hanging a thick black curtain that was attached to the ceiling around the entire circumference of the pool (see below). A square (15 X 15 cm) platform served as

the refuge from the water. The platform could be located either 2.5 cm above (visible task) or 2.5 cm below the surface of the water (hidden task). To increase the prominence of the platform in the visible task, a brightly coloured toy was affixed to its top. The water was made opaque by the addition of floating white polypropylene pellets [7] and maintained at 29°C. Between training trials the animals were placed beneath a heat lamp to reduce core temperature loss. Data were collected using a video-camera that was recessed in the ceiling above the pool and swim trials were digitized, stored on disk, and analyzed using the Poly-Track video system and software (San Diego Instruments).

2.3 2 Beam task

A long narrow wooden beam (1.8cm wide and 86cm long) served to assess motoric performance. A brightly illuminated quartz halogen lamp (1000w) located at the beginning of the beam served to facilitate the rats' traverse to a goal box located at the opposite end. Other than the light from the halogen lamp the room was unilluminated.

2.4 Behavioural testing procedure

2.4.1 Nonspatial pretraining

The NSP regimen employed by Morris [22] was used with some groups (see Table 1). Briefly, a thick black curtain was placed around the circumference of the pool to occlude distal cues. Each rat was given three trials (4 hr intertrial interval) on each of four consecutive days (total trials $n=12$). The location of the hidden platform and the release point varied pseudorandomly from trial to trial. On each trial the rat was allowed to swim until it either mounted the platform or 120s had elapsed (default). Once the rat either found the platform or was manually guided to it (default), it was allowed to remain there

for 30s. No injections were given before or during NSP.

2.4.2 Water maze task

The water maze task was divided into hidden and visible platform components. The hidden task was completed first and was immediately followed by the visible task. All water maze training occurred within the same testing session. Hidden platform training consisted of five blocks of two trials (total trials = 10; inter-trial interval $\approx$ 5 min). A trial was begun by placing a rat in the water next to and facing the pool wall. A trial ended when the rat either mounted the platform or a 60s default time had elapsed. Once the rat either found the platform or was manually guided to it (default), it was allowed to remain there for 15s. The hidden platform was located in the S.E. quadrant of the pool and the trial start positions were pseudorandomly assigned from each of the four cardinal compass points. The trial start positions were assigned so that each training block contained a randomly determined release point that was either close to or away from the submerged platform. Upon completion of hidden platform training a 60s probe trial was given in which each rat was allowed to swim freely with the platform removed.

The visible platform task was performed to document the occurrence of possible sensorimotor dysfunction during testing [20,21]. Training in the visible task was similar to the hidden task (total trials = 10, inter-trial interval $\approx$ 2 min) except that the rats were released from a fixed point (N) and the location of the submerged platform was varied pseudorandomly from trial to trial.

2.4.3 Beam task

The beam task (BT) was used because it has been shown to be a sensitive test of

sensorimotor dysfunction [6]. The subjects used in the BT consisted of the Saline and Gum Arabic controls used in the water maze experiment and additional naive rats of similar weight. The subjects were randomly assigned to one of the following groups: 1. BT PCPA+SCO group (n=5), 2. BT PCPA group (n=8), 3. BT SCO group (n=9), 4. BT Saline group (n=4), and 5. BT Gum Arabic group (n=4). Prior to drug administration the animals were pretrained on the B.T until they had reached an asymptotic level of performance. After the initial pretraining was completed the same drug dose and administration protocol as employed in the water maze experiment was performed. At the time of beam testing the animals weighed between 650-800g. Each rat was given 10 consecutive training trials. If the rat did not traverse the beam within 60s (default) it was removed from the beam and given a short time out before the next trial was begun. If the rat fell off the beam the default score and procedure was initiated. Time to traverse the beam was recorded with a stopwatch.

2.5 Behavioural measures

The following measures were obtained for the hidden platform task: a) hidden platform search time, b) direct swims, defined as a swim path contained completely within an 18 cm conceptual alley [48], c) the percentage of platform contacts that were deflections, defined as an altered swim trajectory caused by contact with the platform; or swimovers, defined as a swim trajectory that went over the length of the platform without pause, d) swim speed, which was calculated by dividing the total distance swum by each rat, over the 10 hidden platform training trials, by each rat's summed search time, e) percent of time in the periphery, defined as the percentage of time the rat spent swimming in the outer 52% of

the pool, f) percent of time in the platform ring, defined as the percentage of time the rat spent swimming in the doughnut shaped inner 35% part of the pool, that was adjacent to the periphery, and which contained all possible platform positions, and g) post-training probe trial. Two separate probe trial measures were evaluated. In the first, the time each rat spent swimming in the quadrant of the pool that had previously contained the platform was measured. In the second, the time each rat spent swimming in the half of the pool that previously contained the platform was measured. For measures (e-g) see appendix II for a descriptive diagram of the various pool divisions. The following measures were obtained in the visible platform task: a) visible platform search time, and b) the total number of loops, defined as a swim trajectory that turned back and crossed itself; and passes, defined as a swim trajectory that did not contact the platform but came within 18 cm of it. The following measures were obtained in the beam task: a) traverse time and b) slips and falls. A slip was scored if both of the animal's hind limbs lost contact with the same side of the beam.

2.6 Statistical analyses

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS/PC[®], version 4.0). A split-plot analysis of variance (ANOVA) was used to analyze platform search data, with group serving as a between subject factor and trial serving as a within subject factor. To avoid problems with heterogeneity of variance, data from the hidden and visible platform tasks were analyzed separately. All remaining data were analyzed with independent groups ANOVA, *t*-tests, and Pearson product-moment correlation coefficients. Posthoc analyses were performed using Student-Newman-Keul's

(SNK) procedure, with $\alpha=.05$ considered as significant.

3. Results

3.1 Water maze

3.1.1 Hidden platform search time

The Saline and Gum Arabic controls, hereafter referred to as Combined Controls, did not differ on any measure and thus were combined for statistical analysis. Figure 1A displays the total hidden platform search time as a function of trial block and group. The Naive PCPA+SCO, the NSP PCPA+SCO, and the Naive SCO groups did not differ, but did have longer search times than the remaining groups, which did not differ ($F(6,69)=15.61$, $p<.001$; Naive PCPA+SCO, NSP PCPA+SCO, Naive SCO vs. each other group, $p<.05$).

Due to the relative insensitivity of the interaction term in ANOVA [32,43], interaction and trial effects were examined with two separate ANOVA performed on the groups clustered about the main effect of Group (Fig 1A). The first ANOVA included the Naive PCPA+SCO, the NSP PCPA+SCO, and the Naive SCO groups. As expected, there was no main effect of group, but there was a significant main effect of trial ($F(9,279)=2.73$, $p<.05$) and a significant group by trial interaction ($F(18,279)=1.74$, $p<.05$). Simple effects analysis indicated that the NSP PCPA+SCO group (SNK, $p<.05$) and the Naive SCO group (SNK, $p<.001$) demonstrated a significant decrease in search time over trials, whereas the Naive PCPA+SCO group did not. The second ANOVA included the Combined Controls, the Naive PCPA, NSP PCPA, and the NSP SCO groups, and revealed only a significant main effect of trial ($F(9,306)=8.58$, $p<.001$). Simple effects

analysis indicated that each of these groups (SNK, $p < .05$) demonstrated a significant decrease in search times across trials.

3.1.2 Direct swims

The Naive PCPA+SCO, the NSP PCPA+SCO, and the Naive SCO groups did not differ, but had fewer direct swims than the other groups, which did not differ ($F(6,69)=5.17$, $p < .001$; Naive PCPA+SCO, NSP PCPA+SCO, and Naive SCO vs. each other group, $p < .05$; Fig 1B).

3.1.3 Post-training probe trial

The Combined Controls, Naive PCPA, NSP PCPA, and the NSP SCO groups did not differ and spent significantly more time swimming in the platform quadrant than the remaining groups, which did not differ ($F(6,69)=7.91$, $p < .0001$, Combined Controls, Naive PCPA, NSP PCPA, and NSP SCO vs. each other group $p < .05$; Fig 1C). The half-pool probe trial analysis revealed that all groups except the Naive PCPA+SCO group spent significantly more time swimming in the half of the pool that previously contained the platform (all groups except Naive PCPA+SCO, $p < .05$; paired t -tests: data not shown).

3.1.4 Percent of time in periphery and platform ring

The Naive PCPA+SCO, NSP PCPA+SCO, and Naive SCO groups did not differ and spent more time swimming in the periphery of the pool and less in the platform ring than the other groups, which did not differ (periphery: $F(6,69)=12.31$, $p < .0001$; platform ring: $F(6,69)=14.09$, $p < .0001$; Naive PCPA+SCO, NSP PCPA+SCO, Naive SCO vs. each other group, $p < .05$; Fig 2A).

Fig. 1. Water maze acquisition. (A) Hidden and visible platform search time as a function of trial block and group. (B) Mean percentage of direct swims to the hidden platform. (C) Platform quadrant dwell time in the hidden platform post-training probe trial. The dashed line (15s) represents chance performance (for key see 1B). In this and the following figures data points represent means and error bars represent S.E.M. PCPA = p-chlorophenylalanine; SCO = scopolamine hydrobromide; NSP = nonspatial pretraining.

1A

Water Maze Trial Blocks

13

1B

Direct Swims

1C

Probe Trial

Fig. 2. Distribution of swim time and measures of sensorimotor performance. (A) Percentage of hidden platform swim time in the periphery and platform ring. (B) Percentage of platform contacts that were deflections and swimovers in the hidden platform task (for key see 2A). (C) Total number of loops and passes in the visible platform task (for key see 2A). PCPA = p-chlorophenylalanine; SCO = scopolamine hydrobromide; NSP = nonspatial pretraining.

Percent of Contacts That Were
Deflections and Swimovers

Loops & Passes

3.1.5 Platform contacts that were deflections and swimovers

Due to the considerable within group variability associated with this measure only a nonsignificant trend for a main effect of group was observed ($F(6,69)=1.94$, $p=.08$; Fig 2B). However, based on previous research [29], we expected that the Naive SCO group would have more deflections and swimovers than the NSP SCO group. Therefore, a one tailed a priori t -test (using the error term from the ANOVA) was performed. The analysis confirmed our expectation that the Naive SCO group had significantly more platform contacts that were deflections and swimovers than the NSP SCO group ($t(69)=1.68$, $p<.05$).

3.1.6 Swim speed

There were no significant group differences in swim speed ($F(6,69)=1.87$, $p>.05$; data not shown).

3.1.7 Visible platform search time

The Naive PCPA+SCO group had longer visible platform search times than the other groups, which did not differ ($F(6,69)=6.17$, $p<.001$; Naive PCPA+SCO vs. each other group, $p<.05$; Fig 1A). The ANOVA also revealed a significant main effect of trial ($F(9,621)=5.5$, $p<.001$) and a significant group by trial interaction ($F(9,54)=1.48$, $p<.05$). The basis of the interaction was that the Naive PCPA+SCO and NSP PCPA+SCO groups demonstrated a significant ($p<.05$) decrease in search time across trials, whereas the other groups did not. It is likely that the reason the other groups did not demonstrate this pattern was that they had already reached asymptotic performance early in training (Fig 1A).

Fig. 3. Performance on the beam task. (A) Traverse time. (B) Percent of trials in which there was a slip or fall. BT = beam task; PCPA = p-chlorophenylalanine; SCO = scopolamine hydrobromide.

Beam Task

3.1.8 Visible platform loops and passes

In the visible platform task the Naive PCPA+SCO group had more loops and passes than the remaining groups, which did not differ ($F(6,69)=7.08$, $p<.0001$; Naive PCPA+SCO vs. each other group, $p<.05$; Fig. 2C).

3.2 Beam Task

There were no group differences between the BT Saline and BT Gum Arabic controls; thus the groups were collapsed for statistical analysis and hereafter referred to as BT Combined Controls. The BT Combined Controls had the quickest beam traverse times, the BT PCPA+SCO group had the slowest, and the BT SCO and BT PCPA groups were intermediate between these extremes ($F(3,26)=8.51$, $p<.001$; BT PCPA+SCO>BT SCO & BT PCPA>BT Combined Controls, $p<.05$; Fig. 3A). The BT PCPA+SCO group had the most trials that were slips and falls, the BT Combined Controls had the least, and the BT SCO and BT PCPA were intermediate between these extremes ($F(3,26)=9.44$, $p<.001$; BT PCPA+SCO>BT SCO & BT PCPA>BT Combined Controls, $p<.05$; Fig. 3B).

3.3 Correlations

Table 2 contains the Pearson product-moment correlation coefficients between the various measures used throughout the hidden platform portion of the study. Of particular interest was the finding of a correlation between hidden platform search time, probe trial platform quadrant swim time, and percent direct swims. This finding was relevant because it demonstrated a consistency between the various measures of spatial acquisition. Of further interest was the finding of a correlation between hidden platform search time, percent direct swims, percentage of time in the periphery, and percentage of platform

TABLE 2.
Correlations between water maze hidden platform measures

Measure 1	Measure 2	r	p
Hidden platform search time	Platform quadrant dwell time	-.64	<.001
	% Direct swims	-.70	<.001
	% Time in periphery	.81	<.001
	% Time platform ring	-.80	<.001
% Time in periphery	% Contacts (deflection or swimover)	.55	<.001
	% Time in platform ring	-.97	<.001
	% Direct swims	-.59	<.001
	Platform quadrant dwell time	-.58	<.001
% Direct swims	% Time in platform ring	.57	<.001
	% Contacts (deflection or swimover)	-.36	<.01
	% Platform quadrant dwell time	.45	<.001
	% Time in platform ring	.60	<.001
Platform quadrant dwell time	% Direct swims	.45	<.001
	% Platform quadrant dwell time	.60	<.001

The values represent the product-moment correlation coefficient that resulted when measure 1 was correlated with measure 2. The following groups were included in the analysis: Naive PCPA+SCO; Naive PCPA; Naive SCO; and the Combined Controls. All hidden platform water maze measures (section 2.5) were included in the analysis.

contacts that were deflections or swimovers. These and several other of the correlations reported in Table 2 consistently indicated an association between poor maze acquisition scores and a high incidence of sensorimotor dysfunction.

4. Discussion

In this study a detailed behavioural analysis was used to investigate the effect of NSP on water maze performance in rats subjected to muscarinic, serotonergic, and combined muscarinic and serotonergic antagonism. The combination of a detailed behavioural analysis and NSP was chosen because it has proven useful in clarifying the role of some neurotransmitter receptor populations in the field of learning and memory [1,4,6,28,29]. The major findings of the present study were: a) depletion of serotonin with PCPA had no effect on water maze performance, b) antagonism of muscarinic receptors produced impairments in spatial and sensorimotor function in naive rats but neither effect was observed in rats first given NSP, c) combined disruption of muscarinic and serotonergic function produced a severe impairment in spatial performance and sensorimotor function that was only partially alleviated by NSP, and d) there was an association between poor maze acquisition scores and a high incidence of sensorimotor dysfunction.

The most robust effect of NSP was observed in the rats that received SCO. Previous research has shown that rats treated with anti-muscarinic drugs typically perform poorly in a wide variety of learning and memory tasks [12,14,35,40,44]. Observations such as these have led to the suggestion that cholinergic function is crucial for learning and memory [49]. However, these and earlier findings [29] indicated that rats given NSP and then trained under muscarinic receptor antagonism learned the task as rapidly and effectively as

controls. Thus, these findings do not support the suggestion that cholinergic function is crucial for learning and memory.

A critical question that remains to be answered is: How does NSP alleviate the spatial performance deficit associated with muscarinic receptor antagonism? An examination of the performance of the NSP SCO group and the Naive SCO group, on the various measures of sensorimotor function, yielded striking differences in behaviour that may have contributed to the NSP effect. For example, animals in the Naive SCO group spent nearly 25% more time swimming in the periphery of the pool than the NSP SCO group. In this experiment, swimming in the periphery was non-adaptive because the hidden platform was never located there. Thus, this behaviour would have severely limited the opportunity of the Naive SCO rats to spontaneously encounter the platform, which likely interfered with subsequent maze acquisition [6,25]. The Naive SCO group also had a greater number of platform contacts that were deflections and swimovers. It was clear from the data obtained from the beam task and the water maze performance of the Naive SCO group that, under certain circumstances, muscarinic receptor antagonists can produce a variety of sensorimotor disturbances. However, the present findings have confirmed previous work demonstrating that drugged animals can show improved task performance, via a reduction in sensorimotor dysfunction, if they are first given a sufficient amount of task relevant experience [6,9,15,22,29,31]. Although, the factors that constitute sufficient task-relevant experience are difficult to quantify, ongoing work in our laboratory is directed at fractionating the NSP effect to determine the relative contribution of its various components to subsequent maze acquisition [16]. It is likely that NSP contributes to maze

acquisition through a variety of mechanisms [5]. For instance, the repeated handling and prior familiarization with the testing apparatus might reduce the stress associated with spatial training and result in better performance scores. It has also been suggested that prior experience with the specific motor behaviours and general strategies of the task might serve to facilitate maze acquisition, perhaps through the formation of new synaptic contacts that are important for subsequent maze acquisition [5,45].

Although NSP completely alleviated the spatial performance deficit associated with muscarinic receptor antagonism, it did not completely alleviate the deficit associated with combined muscarinic and serotonergic antagonism. This is the first instance in which NSP has not produced a complete or near-complete alleviation of the deficits associated with a wide variety of drug manipulations [1,4,6,16,30,31]. However, NSP did produce some subtle improvements on three of the five measures of spatial acquisition and one measure of sensorimotor function. For example, in the hidden platform task, the NSP PCPA+SCO group had a small but significant decrease in search time as training progressed, whereas the Naive PCPA+SCO group did not. In addition, when the probe trial was analyzed as the time spent swimming in each half of the pool, all groups, except the Naive PCPA+SCO group spent significantly more time in the half of the pool that previously contained the platform. Finally, the Naive PCPA+SCO group took significantly longer to find the platform and made more loops and passes in the visible platform task than the NSP PCPA+SCO group. In summary, although NSP resulted in a subtle improvement in the performance of the NSP PCPA+SCO group, it was not sufficient to completely alleviate the deficit. The above findings illustrate the utility of employing a detailed

behavioural analysis to water maze performance because without such observations it becomes difficult to accurately infer the amount of learning in animals that have received various forms of experimental manipulation.

There are several possible reasons to account for the failure of NSP to completely alleviate the spatial performance deficit induced by muscarinic and serotonergic antagonism. The first is that the pretraining procedure, as initially designed by Morris [22] and used here, may not have been of sufficient strength to prevent the disruption of performance associated with this particular form of antagonism. It is possible that increasing the duration of the NSP phase or the number of trials per day might have produced a more substantial impact on subsequent maze performance. Future work with this particular form of antagonism should investigate the effect of increasing the strength of the NSP phase to determine if a more rigorous protocol could alleviate the deficits observed here.

As mentioned previously, there is a substantial body of evidence implicating the cholinergic cells of the basal forebrain and the serotonergic cells of the brain stem in providing a form of ascending control over the electrical activity of the hippocampus and neocortex [38,39,42]. Disruption of the cholinergic and serotonergic inputs to these structures can completely abolish spontaneous cerebral activation, producing a condition that earlier work in the field could only achieve by inducing gross damage to the brain stem or diencephalon [37]. In terms of behaviour, there have been several reports which have suggested that combined disruption of cholinergic and serotonergic function results in animals that appear entirely unable to engage in any form of adaptive behaviour

[37,38,39,42]. Although it was clear from the results of the present study that co-administration of PCPA and SCO produced severe impairments in spatial navigation, it remained unclear as to the exact nature of this impairment. For a rat to perform in the water maze it must be able to integrate information acquired from distal cues and respond to them by making the necessary motor adjustments required to navigate to the platform. Therefore, an intact visual and motor system are essential for successful completion of the task. There is evidence to suggest that anti-muscarinic drugs produce subtle impairments in vision that may affect spatial navigation [3,13]. Although there have been no studies that have performed a detailed examination of the visual capabilities of rats treated with both PCPA and SCO, the results from the visible platform task suggest that the Naive PCPA+SCO rats might have experienced a visual impairment. Thus, the poor performance in rats treated with PCPA and SCO might have been attributable to a decrease in visual functioning. In terms of motor performance, the BT PCPA+SCO group clearly exhibited a degree of ataxia. This result was likely do to the disruption of cholinergic and serotonergic inputs to the neocortex, neostriatum, and cerebellum, all of which are structures that have been implicated in controlling motor function. Although the extent to which beam task performance can be generalized to swimming efficacy is not known, when these data are considered along with the high percentage of swimovers and deflections observed in the Naive PCPA+SCO group, it suggests that the motor impairment observed in the beam task was present and relevant to the water maze. Furthermore, in other studies carried out in our laboratory, beam task performance has been shown to correlate with measures of water maze performance such as percentage of

time in the periphery, percentage of platform contacts that were deflections and swimovers, hidden platform search time, and platform quadrant swim time [4,6,16,29].

The finding of numerous sensorimotor disturbances in the animals treated with PCPA and SCO is not surprising in light of what is known about the effect of these neurochemical systems on brain activity and behaviour. In the past, animals that have been treated with this particular drug combination have been described as "demented," with a global impairment in a wide variety behaviours [10,37]. In fact, this preparation produces some of the behavioural phenomena observed in surgically decorticate rats, such as eating inappropriate foods, abnormal nesting habits, and abnormal grooming patterns. [17,37]. These observations are important because they represent species typical behaviours and thus illustrate the pervasiveness of the deficit. It was interesting to note that there were no group differences observed in swimming speed; yet it was clear from the data presented above that several of the groups had experienced a marked degree of sensorimotor dysfunction. Therefore, it appears that swimming speed might be an insensitive measure of sensorimotor dysfunction. This finding is particularly relevant to water maze research because swimming speed has often been employed as the only measure of sensorimotor dysfunction.

Due to the numerous sensorimotor disturbances observed in the rats treated with PCPA and SCO, it becomes difficult, if not impossible, to delineate the exact role of muscarinic and serotonergic systems in spatial learning and memory. In fact, an argument has been made that data of the type presented in this paper do not justify conclusions about the specific role of these systems in learning and memory [37,41]. There is considerable

ambiguity concerning the terms "learning" and "memory." For instance, learning and memory can be defined as an overt change in behaviour (ie. behavioural definition) or a change in central nervous system (CNS) function brought about by environmental experience (ie. synaptic definition; Refs; 37,41). Therefore, the adoption of either a behavioural or synaptic definition of learning and memory will affect the interpretations made by studies of this nature. For example, if it is assumed that the behavioural definition is correct then the results from the present experiment suggest that cholinergic and serotonergic systems are crucial for learning and memory. However, if it is assumed that the synaptic definition is correct then the poor performance displayed by rats treated with PCPA and SCO could be due to either a failure to modify existing synapses, a disruption in CNS function, or both. The synaptic definition is based on the view that learning and memory results in a relatively permanent changes in synaptic function [36]. Thus, before it can be stated that certain experimental manipulations cause impairments that are specific to learning, it must be ruled out that the impairments were not caused by disruption of the behaviours necessary to perform the task. However, as previously mentioned, there is evidence to suggest that rats treated with PCPA and SCO incurred a global impairment in a wide variety of behaviours. Furthermore, this impairment appeared to include both sensory and motor components that likely affected the ability of the rats to perform in the maze. Therefore, on the basis of the present evidence it cannot be determined whether the impairment in spatial navigation was the result of a specific learning deficit, an inability to perform the necessary behaviours required to complete the task, or both.

Appendix II

In "A" the template used for calculating time spent in the pool ring and periphery of the pool. In "B" the template used for calculating time spent in the platform quadrant. In "C" the template used to calculate time in the half-pool.

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