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Combined but not Individual Administration of β-Adrenergic and Serotonergic Antagonists Impairs Water Maze Acquisition in the Rat

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This study examined the effects of serotonergic depletion and β-adrenergic antagonism on performance in both visible platform and hidden platform versions of the water maze task. Male Long–Evans rats received systemic injections of *p*-chlorophenylalanine (500 mg/kg × 2) to deplete serotonin, or propranolol (20 or 40 mg/kg) to antagonize β-adrenergic receptors. Some rats received treatments in combination. To separate strategies learning from spatial learning, half of the rats underwent Morris' water maze strategies pretraining before drug administration and spatial training. Individual depletion of serotonin or antagonism of β-adrenergic receptors caused few or no impairments in either naive or pretrained rats in either version of the task. In contrast, combined depletion of serotonin and antagonism of β-adrenergic receptors impaired naive rats in the visible platform task and impaired both naive and strategies-pretrained rats in the hidden platform task, and also caused sensorimotor impairments. This is the first finding of a 'global' water maze task/sensorimotor impairment with combined administration of two agents that, at the high doses that were given individually, produced few or no impairments. The data imply that (1) serotonergic and β-adrenergic systems may interact in a manner that is important for adaptive behavior; (2) impairments in these systems found in Alzheimer patients may be important for their cognitive and behavioral impairments; and (3) the approach used here can model aspects of the cognitive and behavioral impairments in Alzheimer disease.
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INTRODUCTION

Drug treatments that alter the function of any of a large variety of neurotransmitter systems can impair acquisition of the water maze (WM) task (Bannerman *et al*, 1995; Beiko *et al*, 1997; Cain, 1997, 1998; Cain *et al*, 1996, 1997, 2000; Morris and Inglis, 2003; Morris, 1989; Saucier and Cain, 1995; Saucier *et al*, 1996; Vanderwolf, 1987; Whishaw, 1989; Whishaw and Tomie, 1987). Certain other treatments, such as antagonism of β-adrenergic receptors with propranolol (PRO), destruction of noradrenalin-containing neurons in the locus coeruleus, or depletion of serotonin by *p*-chlorophenylalanine (pCPA), have little or no effect on performance on the WM or radial arm maze (Beatty and Rush, 1983; Beiko *et al*, 1997; Decker *et al*, 1990; Harder *et al*, 1996; Hiraga and Iwasaki, 1984; Richter-Levin and Segal, 1989; Riekkinen *et al*, 1992; Vanderwolf and Baker, 1996). In contrast to treatment with a single drug, combined treatment with two drugs can markedly impair WM performance. Thus, naive rats given either PRO or a low dose of scopolamine, a muscarinic cholinergic antagonist, had WM search times comparable to saline controls, whereas a combination of the two treatments increased WM search times (Decker *et al*, 1990). Similarly, rats given pCPA had no WM impairment but rats given pCPA together with a muscarinic antagonist were more impaired than rats given scopolamine alone (Beiko *et al*, 1997; Richter-Levin and Segal, 1989; Riekkinen *et al*, 1992; Vanderwolf, 1987).

The findings from these experiments are important because they reveal a greater than additive impairing effect on cognition and behavior when the drugs are administered in combination. This suggests that interactions between neurotransmitter systems are significant for the production of adaptive behavior (Decker and McGaugh, 1991). The findings also suggest a link between the cognitive and behavioral impairments that occur in Alzheimer disease and the fact that multiple neurotransmitter systems such as cholinergic, noradrenergic, and serotonergic systems are typically impaired in the brain of Alzheimer patients (Chen *et al*, 2000; Davis *et al*, 1999; Dringenberg, 2000; Francis *et al*, 1999; Lai *et al*, 2002; Reinikainen *et al*, 1990). Our previous studies confirmed that neither PRO nor pCPA given individually impaired WM performance, and that

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scopolamine impaired naive but not WM strategies-pretrained rats (Beiko *et al*, 1997; Saber and Cain, 2003). We also found that combinations of either PRO and scopolamine, or pCPA and scopolamine impaired all aspects of WM performance in both naive and strategies-pretrained rats, and also caused sensorimotor impairments (Beiko *et al*, 1997; Saber and Cain, 2003). These 'global' cognitive/behavioral impairments due to combined antagonism of neurotransmitter systems known to be impaired in Alzheimer disease are consistent with the cognitive/behavioral impairments seen in Alzheimer patients.

Therefore, further study of the cognitive and behavioral effects of combined neurotransmitter system depletion or antagonism seems warranted. The present study examined the effects of single and combined administration of PRO and pCPA. This combination of treatments has not been studied with rats in the WM task. A spatial navigation task was chosen because Alzheimer patients often are unable to make use of landmarks in a familiar environment, wander away from their living quarters, and display repetitive behaviors that appear purposeless (De Deyn *et al*, 1999; Fairburn and Hope, 1988; Teri *et al*, 1988). These symptoms appear to be similar to the spatial navigation impairments and repetitive thigmotaxic swimming behaviors seen in previous studies that used combined administration of agents relevant to the brain neurotransmitter impairments seen in Alzheimer disease (Beiko *et al*, 1997; Cain *et al*, 2000; Saber and Cain, 2003).

EXPERIMENT 1: VISIBLE PLATFORM TASK

Impairments in swimming to a visible platform have been reported in rats with brain lesions outside the visual system (Figure 1; Morris *et al*, 1982; Vanderwolf and Penava, 1992; Cain and Boon, 2003; Cain *et al*, 2006a, 2006b) and in rats given pharmacological treatments (Cain, 1997; Cain *et al*, 2002). Therefore in Experiment 1, rats given PRO or pCPA alone or in combination were tested in a simple swim-to-visible-platform task to evaluate basic swimming and navigation behavior and to document normal navigation behavior in rats for use in determining the optimal behavioral measures for spatial memory. This information was used in Experiment 2 to evaluate the performance of rats given the same pharmacological treatments in a conventional hidden platform WM task. To evaluate the role of prior familiarity with general WM strategies on the performance of rats given drug treatments and tested in a simple swim-to-visible-platform WM task, some rats in Experiment 1 were first given 1 day of training in the visible platform task in the absence of any drug or vehicle injections, followed by another day of testing under drug (Cain, 1997).

MATERIALS AND METHODS

Subjects

Male Long–Evans rats ($n = 80$, Charles River, Canada) weighing 250–400 g were used. The rats were housed in pairs on a 12 : 12 h light–dark cycle (lights on at 0700 hour) with testing during the light phase of the cycle for comparability with previous WM research. Food and water were available *ad lib*. Before testing, all animals were naive to swimming and all behavioral test procedures. To acclimate the rats to handling, they were removed from their cages, placed on a lab cart, and handled for 5 min on each of 3 consecutive days. Rats were randomly allocated to

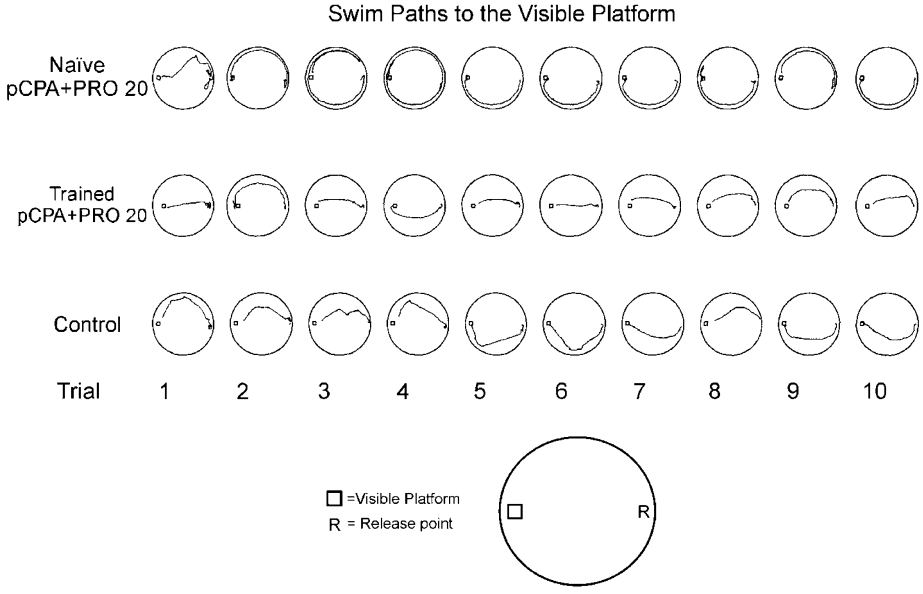

Figure 1 Experiment 1, visible platform task. Individual digitized swim paths on trials 1–10 of the rat in the Naive pCPA + PRO 20, Trained pCPA + PRO 20, and Control groups with the median summed swim time of their respective group. The illustrations at the bottom depict the task, with the release point opposite the visible platform. Each swim progressed from right to left in the figure. There was a 9 cm gap between the wall and the closest edge of the visible platform to prevent rats from bumping into the platform if swimming thigmotaxically. The Naive pCPA + PRO 20 rat was representative of its group in that it swam thigmotaxically and therefore less efficiently than Control rats, whereas the Trained pCPA + PRO 20 rat swam efficiently to the visible platform using a direct or circle swim on most trials. pCPA = *p*-chlorophenylalanine; PRO 20 = propranolol 20 mg/kg.

groups. All animal procedures were in accordance with the Guide for the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

Drug Treatments

The drug treatments were pCPA (Sigma), an inhibitor of serotonergic biosynthesis (Koe and Weissman, 1966; Vanderwolf and Baker, 1996), and propranolol hydrochloride (Sigma), a specific β-adrenergic antagonist. Dose selection was based on published data (see below) and the strict criterion that the treatments not interfere with swimming and climbing behavior in the WM. Drug treatment groups are summarized in Table 1.

pCPA (1000 mg/kg) was suspended in a gum arabic (0.5%)/saline (0.9%) solution and was administered for 2 consecutive days, using a single 500 mg/kg intraperitoneal (i.p.) injection each day. The second injection occurred 3 days before behavioral testing. This dose and injection protocol has been shown to reliably reduce whole-brain levels of both serotonin and 5-hydroxyindoleacetic acid by greater than 90%, with only slight reduction in whole-brain levels of dopamine and noradrenaline (Dringenberg *et al*, 1995; Vanderwolf and Baker, 1996). Another reason for choosing this dose is that when administered in this manner, pCPA produced little or no impairment in WM performance in the rat; however, this dose of pCPA administered together with scopolamine severely impaired WM performance in both naive and strategies-pretrained rats to a significantly greater extent than scopolamine alone (Beiko *et al*, 1997).

PRO (20 or 40 mg/kg) was administered in saline (0.9%; 1.0 ml/kg i.p.) approximately 20 min before behavioral testing on each day of testing. PRO readily crosses the blood–brain barrier and antagonizes both β-1- and β-2 noradrenergic receptors in the thalamus, neocortex, and hippocampus (Booze *et al*, 1989). The 20 mg/kg PRO dose was chosen because it was the threshold dose for minor alterations in open field behavior (eg decrease in rearing; Angrini *et al*, 1998), but when administered by itself 20 mg/kg PRO was without effect on WM or radial arm maze performance (Beatty and Rush, 1983; Hiraga and Iwasaki, 1984; Saber and Cain, 2003). A dose of 20 mg/kg PRO was combined with pCPA treatment, because 20 mg/kg PRO administered together with scopolamine severely impaired WM performance in both naive and strategies-pretrained rats (Saber and Cain, 2003), which suggests that this dose is relevant for studying the effects of drug combinations that include PRO. A combination of 40 mg/kg PRO and pCPA was not used here, because WM performance was reported to be unaffected by a dose of 80 mg/kg of PRO (Skinner *et al*, 1996) and because the data showed that a dose of 20 mg/kg PRO combined with pCPA was adequate to severely impair performance on both the visible and hidden platform versions of the WM (see below). We previously found that nadolol administered together with scopolamine as a control for peripheral effects of β-adrenergic antagonism produced no WM impairments in strategies-pretrained rats and only limited impairments that were confined to the earlier part of behavioral training in naive rats (Saber and Cain, 2003). This suggests that when PRO impairs WM performance, it does so by acting on the central nervous system.

Table 1 Groups Studied and Drugs Administered in Experiment 1

Group	Treatment	Dose	*n*
Naive pCPA	pCPA	1000 mg/kg	8
Naive PRO 20	PRO	20 mg/kg	8
Naive PRO 40	PRO	40 mg/kg	8
Naive pCPA+PRO 20	pCPA, PRO	1000 mg/kg, 20 mg/kg	8
Naive Control	Gum arabic, saline	10 mg/kg, 1 ml/kg	8
Trained pCPA	pCPA	1000 mg/kg	8
Trained 20 PRO	PRO	20 mg/kg	8
Trained 40 PRO	PRO	40 mg/kg	8
Trained pCPA+PRO 20	pCPA, PRO	1000 mg/kg, 20 mg/kg	8
Trained Control	Gum arabic, saline	10 mg/kg, 1 ml/kg	8

pCPA = parachlorophelylalanine; PRO = propranolol hydrochloride; *n* = number of rats in group.
pCPA or gum arabic vehicle were administered on 2 consecutive days at 500 mg/kg per day, with the second injection given 3 days before the start of behavioral testing. PRO or saline vehicle was administered approximately 20 min before behavioral testing on each day of testing.

Apparatus

The WM was a white circular pool (1.5 m diameter) located in the center of a large room with numerous visual cues (doors, cabinets, posters on the walls, etc.). The intensity of illumination 1 cm above the surface of the water at the center of the pool was 320 lux. The refuge was a visible platform (15 × 15 cm) that protruded 2 cm above the surface of the water (29 ± 1°C) and was marked by a cylindrical object 3 cm in diameter and 10 cm tall. For maximum visibility, both the platform sides (as viewed by the rat from water level) and the cylindrical object on the visible platform were painted in alternating black and white stripes at a spatial frequency that is well within the visual acuity of the Long–Evans rat (Prusky *et al*, 2002). The platform was placed close to the pool wall, opposite to the start point of each swim, to provide a simple task that required the rat to swim directly across the pool from a stable start point to a stable visible refuge (see Figure 1). There was a 9 cm gap between the wall and the closest edge of the visible platform to prevent rats from simply bumping into the platform if swimming thigmotaxically and for consistency with previous use of this task (Cain and Boon, 2003; Cain *et al*, 2006a, b). The same white polypropylene pellets that were used to render the surface of the water opaque in Experiment 2 (Cain *et al*, 1993) were used in this experiment to make swimming conditions comparable. Rats were placed under a heat lamp between trials to maintain core body temperature. The signal from a video camera recessed into the ceiling above the center of the pool was sent to a VCR and a tracking system (Poly-Track, San Diego Instruments) that produced videotaped records and a digital files of all swim trials. These were later objectively analyzed during the detailed behavioral analysis.

Testing Procedures and Behavioral Analysis

Visible platform training. Half of the rats ($n = 40$) were initially trained on the visible platform task in the absence of any drug or vehicle injections and are referred to as the Trained groups (see Table 1). This initial training was given to familiarize the rats with the pool and with general WM strategies before they received drug treatments. The training consisted of 10 trials (5 min intertrial interval) given in 1 day. Rats were released one at a time into the water immediately adjacent to and facing the pool wall opposite the visible platform (see Figure 1). The distance from the start point to the closest edge of the visible platform was 128 cm. After the rat climbed onto the visible platform, it remained there for 15 s. The experimenter was not visible to the rat while it was swimming, and the swims were viewed on a video monitor situated outside the WM room.

Visible platform testing. Visible platform WM testing began 5 days after the completion of initial visible platform training, if given. Before visible platform testing, each rat received the appropriate treatment as indicated in Table 1. The visible platform testing procedure was identical to the visible platform training procedure described above, with each rat receiving one session of testing (10 trials, 5 min intertrial interval). Squads of 4–6 animals were tested in each session to ensure that all testing was completed within 1 h while the drug treatments were maximally effective. The lights in the WM room were illuminated, with all distal cues available and the experimenter not visible to the rat while it swam in the pool.

Detailed behavioral analysis. Digital files of trials were objectively analyzed for two measures of task acquisition, visible platform search time, and direct and circle swims. Visible platform search time was the time from release into the pool until the rat mounted the platform. The criterion for a direct swim was that the rat remains within an 18-cm-wide virtual 'alley' from the start point to the platform (Whishaw and Tomie, 1987). A circle swim was a swim trajectory that approximated an arc of a circle from the start point to the platform without exceeding 360°of circling or crossing over itself (Whishaw and Jerrard, 1995). A thigmotaxic swim in which the rat was in contact with the pool wall was not scored as a circle swim because this was considered an inefficient means of reaching the visible platform that was mainly guided by the pool wall. Direct and circle swims were analyzed because they describe the swim paths that normal rats take in this task (Cain and Boon, 2003; Cain *et al*, 2006a, b) and because they are the most stringent measure of spatial memory available for this task (Whishaw and Jerrard, 1995; Whishaw *et al*, 1995).

Statistical analyses. Repeated measures analysis of variance (ANOVA; SPSS) with group as the between-subject factor and trial as the within-subject factor was used to analyze the search time data. For all within-subject effects, degrees of freedom were reduced using the Greenhouse-Geisser epsilon multiplier. One-way ANOVA was used to analyse efficient swims, and *post hoc* analyses were conducted using Dunnett's tests where appropriate. $p \leqslant 0.05$ were considered significant.

RESULTS

All rats displayed normal swimming behavior, with the expected forelimb inhibition and alternate thrusting of the hindlimbs, and made use of the visible platform as a refuge. As expected, naive pCPA rats resisted capture and vocalized when first placed into the water, confirming earlier reports of reactivity to novel stimuli (Brody, 1970; Dringenberg *et al*, 1995). Their initial behavior in water usually consisted of rapid water-treading movements followed by rapid initiation of swimming. As shown in Figure 1, representative swim paths of the rat in each double-drug group with the median summed swim time of its group for the 10 trials indicate differences between some groups in the swim paths taken to the visible platform. The most obvious group difference was the tendency of the Naive pCPA + PRO 20 rat to generate strongly thigmotaxic swim paths *vs* the tendency of the Trained pCPA + PRO 20 rat to generate relatively direct and efficient swim paths.

This impression was confirmed by analysis of the group mean swim time data, which for clarity of presentation are plotted as blocks of two trials each, separately for Naive and Trained groups in Figure 2a and b respectively. Preliminary analysis indicated that the Naive Control and Trained Control groups did not differ on any measure ($p > 0.05$), and thus were combined into a single Control group for subsequent analyses. Statistical analysis using all groups in a repeated measures ANOVA with treatment group as the between groups factor and trial as the within groups factor yielded a significant main effect of Group ($F(8, 71) = 4.00$, $p < 0.001$). *Post hoc* comparisons indicated that the Naive pCPA + PRO 20 group had significantly longer search times than the Control group ($p < 0.01$) but that no other groups differed. The analysis also yielded a significant main effect of Trial ($F(5, 366) = 37.79$, $p < 0.0001$) and a significant Group × Trial interaction ($F(41, 366) = 2.43$, $p < 0.0001$). These analyses indicate that the Naive pCPA + PRO 20 group was impaired relative to Control group, but that the Trained pCPA + PRO 20 group was not impaired. The analysis also suggested that the groups improved during the course of testing, but that some groups improved more than others as testing progressed.

The performance of the groups on the direct and circle swim measure appeared consistent with the above findings, with the Control and Trained groups generally producing direct or circle swims. As shown in Figure 2c, the Naive pCPA + PRO 20 group performed poorly relative to all of the other groups. This impression was confirmed by ANOVA results indicating a significant effect of group ($F(8, 71) = 3.76$, $p < 0.01$). *Post hoc* tests indicated that the Naive pCPA + PRO 20 group had a significantly lower percentage of direct and circle swims than Control group ($p < 0.001$).

DISCUSSION

Neither individual serotonergic depletion nor β-adrenergic antagonism impaired performance in either naive or trained rats. These results are consistent with earlier reports of no effect of these treatments on performance of either visible platform or hidden platform WM tasks or the radial arm maze task (Beatty and Rush, 1983; Beiko *et al*, 1997; Decker

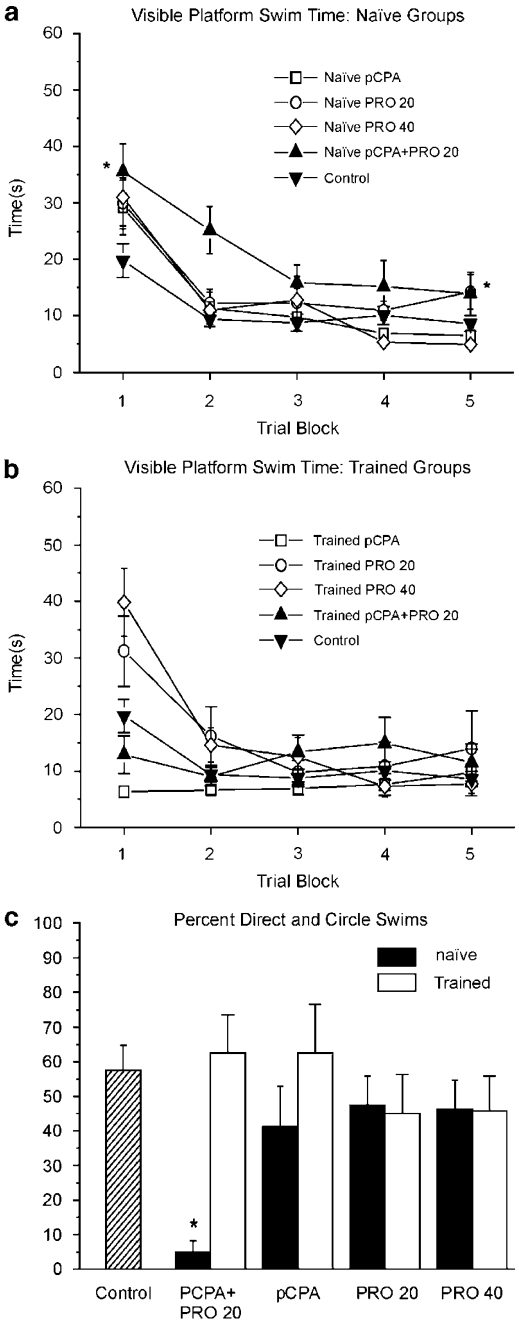

Figure 2 Experiment 1, visible platform task. Group mean swim time per trial for the Naive groups (a) and the trained groups (b), and group mean percent direct and circle swims for all groups (c). For clarity of presentation here and in Figures 3 and 4, data in (a) and (b) are graphed as trial blocks, where each block represents the mean of two trials. Statistical analysis made use of data from all individual trials. The Naive pCPA + PRO 20 group was impaired on both measures. *Indicates values significantly different from controls at $p<0.05$ or better (see text). In this and the following figures, data points or histogram bars represent group means, and error bars represent $\pm$SEM, plots within 1 day that differ significantly from other plots are indicated by symbols next to the first and last symbols of the plot; and for data point symbols that do not have error bars, the bars are contained entirely within the symbols. pCPA = *p*-chlorophenylalanine; PRO 20 = propranolol 20 mg/kg; PRO 40 = propranolol 40 mg/kg.

et al, 1990; Harder *et al*, 1996; Hiraga and Iwasaki, 1984; Saber and Cain, 2003; Richter-Levin and Segal, 1989; Riekkinen *et al*, 1992; Vanderwolf, 1987). However, despite the extreme simplicity of the task, and with no impairment in swimming behavior as such, the Naive pCPA + PRO 20 group was clearly impaired in the task, especially in swim path efficiency as measured by direct and circle swims. In contrast, when given the same treatments, the Trained pCPA + PRO 20 group performed as well as Control group. This finding, together with the significant Trial and Group × Trial interaction effects, suggests that there are important issues of strategies learning and perhaps also stress reduction (Beiko *et al*, 2004) that can affect how animals perform even the simplest swim-to-visible platform task. Similar conclusions have emerged from work using other drug treatments or brain lesions (Cain, 1997; Cain and Boon, 2003; Cain *et al*, 2002, 2006a, b).

Several observations suggest that the combined treatments in the Naive pCPA + PRO 20 group may not have resulted from a visual impairment in guiding swims to the visible platform. These include: (1) swims ended at the visible platform with no local searching for the platform on nearly every trial, suggesting that rats could visually detect the platform; (2) no rat in either pCPA + PRO group was ever seen to walk off the edge of the transport cart or bump into objects or other rats; (3) brain lesions that do not damage the visual system also produce impairments comparable to those found here in a visible platform WM task (Cain and Boon, 2003; Cain *et al*, 2006a, b; Figure 1 in Morris *et al*, 1982); (4) the Trained pCPA + PRO 20 group effectively used visual cues to navigate to the visible platform as efficiently as Control group.

The swim paths in Figure 1 indicate that, in addition to producing direct errorless swims to a refuge (strictly defined as being contained entirely within a virtual 18 cm-wide alley from the start point to the refuge; Harker and Whishaw, 2002; Kolb *et al*, 1994), naive control rats frequently produce curved swims that extend into the periphery area of the pool even when swims are started from the same place on every trial and always end at a stable visible refuge. The significant effect of Trial in the statistical analysis suggests that rats improve in task-relevant navigation behaviors even for this simplest version of the WM. These results imply that rats normally generate a variety of efficient swim trajectories in navigating in a pool, some of which are direct swims as previously defined (Harker and Whishaw, 2002; Kolb *et al*, 1994), and some of which approximate an arc of a circle, as shown in Figure 1. These facts have been taken into consideration in adopting a direct and circle swim measure of spatial memory that is used in Experiment 2, along with strategies-pretrained Control groups for separation of WM strategies training and spatial training (Morris, 1989).

EXPERIMENT 2: SPATIAL LEARNING TASK

Experiment 2 examined the effect of β-adrenergic blockade and serotonergic depletion individually and in combination, on acquisition of a conventional WM task, with a hidden platform. Some groups contained rats given WM strategies pretraining (hereafter, pretraining) before drug treatment to separate the strategies learning and spatial learning components of the task (Bannerman *et al*, 1995; Morris, 1989; Morris and Inglis, 2003). Pretraining familiarized the

rats with the general behavioral strategies required in the WM task (eg swimming; suppressing the instinctive response to swim thigmotaxically near the pool wall; recognizing and using the hidden platform as a refuge; Cain, 1998; Whishaw, 1989) without training them to find a specific hidden platform location at this stage of the experiment. Previous work has shown that some drug treatments cause sensorimotor disturbances that could be relevant to performance in the WM task (Beiko *et al*, 1997; Cain, 1997, 1998; Cain *et al*, 1996, 1997, 2000, 2002; Hoh *et al*, 1999; Saber and Cain, 2003; Saucier and Cain, 1995; Saucier *et al*, 1996). Therefore rats were also tested on a beam walking task (Kolb and Whishaw, 1985) that assessed sensorimotor function outside the WM environment under the same drug treatment that was given to the rats before WM training.

MATERIALS AND METHODS

Subjects and Drug Treatments

Male Long–Evans rats ($n = 80$, Charles River, Canada) weighing 250–400 g were used and were housed and tested under the same conditions described for Experiment 1. All rats were naive to experimentation at the start of the experiment and no rats from Experiment 1 were used here. Half of the rats were given Morris (1989) WM strategies pretraining before any drug treatment to familiarize them with the behavioral strategies required in the task. Given the lack of impairment in any group given PRO alone and the clear impairment of the Naive pCPA + PRO 20 group in the visible platform task in Experiment 1, the same drug and control treatments used in Experiment 1 were used here. Rats were randomly allocated to the following groups: Naive pCPA ($n = 8$), Naive PRO 20 ($n = 8$), Naive PRO 40 ($n = 8$), Naive pCPA + PRO 20 ($n = 8$), Naive Control ($n = 8$), Pretrained pCPA ($n = 8$), Pretrained PRO 20 ($n = 8$), Pretrained PRO 40 ($n = 8$), Pretrained pCPA + PRO 20 ($n = 8$), and Pretrained Control ($n = 8$).

Apparatus

The same pool, testing room, and recording/digital tracking system used in Experiment 1 were used here. A uniformly white hidden platform (11×11 cm) with serrations on top for gripping was used for both pretraining and spatial training, with the water surface 2 cm above the top of the platform at all times. The center of the hidden platform was 37.5 cm away from the pool wall, midway between the geometric center of the pool and the pool wall. Floating white polypropylene pellets prevented rats from seeing the platform directly (Cain *et al*, 1993). Thick black curtains on a track attached to the ceiling were drawn completely around the pool during pretraining only to occlude visual cues in the room. For spatial training, the curtains were removed, allowing the rats to make use of visual cues in the room. The room contained lights placed symmetrically into the ceiling directly above the pool and elsewhere in the room to illuminate the pool adequately during both spatial training and pretraining.

Beam task. A wide wooden practice beam (6 cm wide and 86 cm long), located 1 m above the floor, was first used to habituate rats to the procedure in the absence of drug treatment. A narrow wooden beam (1.8 cm wide and 86 cm long), located 1 m above the floor was used to measure sensorimotor coordination after administration of drug or control treatments. A halogen quartz lamp (1000 W) could be positioned at the start of the beam. At the end of the beam was a dark goal area (50×50 cm) that was covered with woodchip bedding. On the floor below the beam was a large bin of woodchips, providing a soft surface if the rats fell.

Testing Procedures and Behavioral Analysis

Beam task. All rats underwent beam task familiarization beginning on experimental Day 1, before any drug treatment. During familiarization on Day 1, ceiling lights illuminated the test room and the halogen lamp was turned off. Each rat was first allowed to traverse the wide beam five times to become familiar with the task. Each rat then traversed the narrow beam 10 times or until it traversed the beam without slipping or pausing for long periods of time. Formal beam task testing was carried out on experimental Day 12, after all WM testing was complete. Each rat received the same drug or control treatments that they received on WM testing days and then underwent 10 consecutive beam task trials with the narrow beam. The halogen lamp illuminated the start end of the beam and the ceiling lights were off. On each trial, the rat was placed under the halogen lamp at the start end of the beam and was given 60 s to reach the goal box at the far end of the beam. Time to traverse the beam was measured using a stopwatch and the number of slips and falls from the beam were recorded. A slip was scored when a hindlimb lost contact with the beam. If a rat fell off the beam, a default 60 s traverse time was recorded.

Strategies pretraining. Half of the rats ($n = 40$) received pretraining on days 2–5 in the absence of drug or control treatments. Pretraining consisted of three trials per day on each of 4 days for a total of 12 pretraining trials, with a 5 min intertrial interval (Morris, 1989). For pretraining, thick black curtains were attached to a circular track fixed to the ceiling and were drawn completely around the pool, eliminating all visual cues from the room. The visible portion of the ceiling above the pool provided no directional cues. The hidden platform was moved to a new quadrant after every pretraining trial. A rat was introduced into the pool and swam until it found the hidden platform or 120 s elapsed, at which time it was placed on the platform where it remained for 30 s. Swimming was observed on the television monitor, and search times were recorded; the experimenter was not visible to the swimming rat. The acquisition of WM behavioral strategies during pretraining has been documented (Hoh *et al*, 1999; Morris, 1989; Perrot-Sinal *et al*, 1996).

Spatial training. Spatial training began on day 10, on the 3rd day after completion of the injections of pCPA or gum arabic, if given, and 20 min after injection of PRO or saline, if given. The black curtain was not present, allowing the rats

to make full use of the distal cues in the room to guide navigation. Rats were tested in squads of four to ensure an adequate intertrial interval of 5 min and the completion of testing in less than 1 h while PRO was maximally effective. Only one rat swam in the pool at a time. Each rat was given 10 trials to find the hidden platform, with the platform fixed in the center of the southeast quadrant. Rats were introduced into the water adjacent to and facing the pool wall at north, south, east, or west, and swam for a maximum of 60 s or until they found and climbed onto the platform. If a rat did not find the platform during a trial, it was manually guided there, where it remained for 15 s. The order of release points was pseudorandomized subject to the constraint that summed distances from the start points to the platform in each two-trial block were approximately equal across blocks. The experimenter monitored each trial on a television screen and was not visible to the rats during the trials. Rats were placed under a heat lamp between trials to avoid loss in body core temperature.

Reversal training. On day 11, rats received reversal training, with the hidden platform in the center of the northwest quadrant, diametrically opposite to the hidden platform location during spatial training. Treatment was again administered as appropriate 20 min before the start of testing, and testing followed the same protocol as on Day 10.

Detailed behavioral analysis. Digital files of trials were objectively analyzed for two measures of task acquisition as described in Experiment 1: hidden platform search time and direct and circle swims.

Use of WM strategies was evaluated by measuring swim time in the pool periphery based on the digitized swim paths obtained using PolyTrak. Swim time in the periphery was the time swum in the outer 50% of the pool area, within 20 cm of the pool wall, where the platform was never located at any time during the study. This was analyzed because swimming away from the pool wall to search the inner region of the pool is an essential strategy in this task, and has a major impact on the search time measure of acquisition (Morris, 1989; Schenk and Morris, 1985).

Statistical analyses. Statistical analyses were as described for Experiment 1, with one-way ANOVA used to analyse beam task data, and *post hoc* analyses conducted using Dunnett's tests where appropriate. Pearson product moment correlations were used to examine relationships between behavioral measures.

RESULTS

Strategies Pretraining

Mean search times to find the hidden platform were similar for all groups, and decreased from a mean of 63.8 s on the first two pretraining trials to a mean of 27.8 s on the last two pretraining trials. This reduction is comparable to reductions found in previous research using the same equipment, pretraining protocol, and rat strain (Cain, 1997; Cain *et al*, 1996, 1997, 2000; Hoh *et al*, 1999; Perrot-Sinal *et al*, 1996; Saucier *et al*, 1996). As pretraining progressed, rats swam away from the wall and readily used the hidden platform as a refuge. These observations indicate that the pretrained rats acquired and made use of the strategies necessary in the task.

Spatial Training

As in Experiment 1, all rats displayed normal swimming behavior and made use of the hidden platform as a refuge when they contacted it. Naive pCPA rats resisted capture and vocalized when first placed into the water, followed by rapid initiation of swimming. The Naive Control and Pretrained Control rats performed similarly on all measures ($p > 0.05$), and thus were combined into a single Control group for all subsequent analyses. Search time and periphery time data for Naive and Pretrained groups are plotted separately for clarity of presentation in Figure 3a and b; however, ANOVA for each measure was conducted with data from all groups.

Search time. As shown in Figure 3a and b, both the Naive and Pretrained pCPA + PRO 20 groups were impaired in search time relative to all other groups. This impression was confirmed by analysis that revealed a significant main effect of Group ($F(8,71) = 10.72$, $p = 0.0001$), with *post hoc* comparisons indicating that both the Naive and Pretrained pCPA + PRO 20 groups and the Naive PRO 40 group had longer search times than Control group ($p < 0.0001$, $p < 0.001$, and $p < 0.01$ respectively), but no other group differences. The analysis also indicated a significant main effect of Trial ($F(7,467) = 54.96$, $p < 0.0001$) and a significant Group × Trial interaction ($F(53,467) = 1.70$, $p < 0.01$) suggesting that the groups improved during the course of testing, but that some groups improved more than others as testing progressed.

Direct and circle swims. Analysis of the direct and circle swim data in Figure 3c by one-way ANOVA confirmed the impression that the Naive pCPA group and the Naive and Pretrained pCPA + PRO 20 groups were impaired (Group: $F(8,71) = 4.79$, $p < 0.0001$; Naive pCPA *vs* Control group, $p < 0.05$; Naive pCPA + PRO 20 and Pretrained pCPA + PRO 20 groups *vs* Control group, $p < 0.001$ and $p < 0.01$, respectively).

WM strategies. As shown in Figure 3a and b, both the Naive and Pretrained pCPA + PRO 20 groups were impaired in WM strategies. Analysis confirmed this impression by revealing a significant main effect of Group ($F(8,71) = 28.84$, $p = 0.0001$), and *post hoc* comparisons indicated that the Naive pCPA group and the Naive and Pretrained pCPA + PRO 20 groups swam significantly more in the pool periphery than the Control group ($p < 0.01$, $p < 0.0001$, and $p < 0.001$ respectively), but that no other groups differed from Control group. Analysis also indicated a significant main effect of Trial ($F(5,357) = 63.99$, $p < 0.0001$) and a significant Group × Trial interaction ($F(40,357) = 6.19$, $p < 0.0001$) suggesting that the groups improved during the course of testing, but that some groups improved more than others as testing progressed.

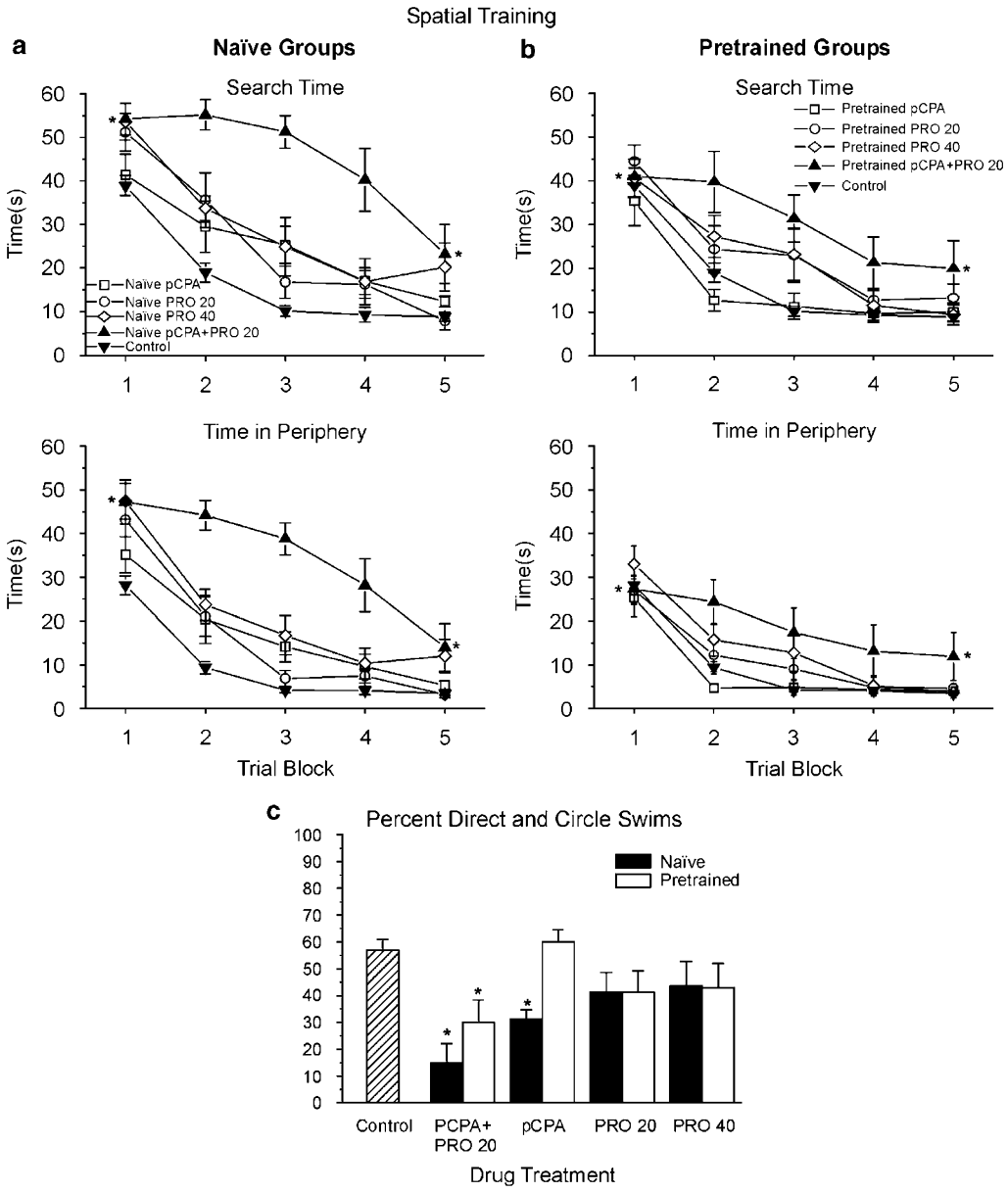

Figure 3 Experiment 2, spatial training. Hidden platform search time and time swum in the pool periphery by Naive groups (a) and by Pretrained groups (b); group mean percent direct and circle swims (c). The Naive and Pretrained pCPA + PRO 20 groups were impaired on all measures. The Naive pCPA group was impaired on the direct and circle swim measure. *Indicates values significantly different from controls, $p<0.05$ or better (see text). pCPA = *p*-chlorophenylalanine; PRO 20 = propranolol 20 mg/kg.

Reversal Training

Search time and periphery time data for Naive and Pretrained groups are plotted separately for clarity of presentation in Figure 4a and b; however, ANOVA for each measure was conducted with data from all groups.

Search time. As shown in Figure 4a and b, the Naive pCPA + PRO 20 group was impaired in search time, an impression confirmed by analysis that revealed a significant main effect of Group ($F(8,71)=2.67$, $p=0.05$). *Post hoc* comparisons indicated a difference between the Naive pCPA + PRO 20 group and Control group ($p<0.05$), but no other group differences. There was also a significant main effect of Trial ($F(5,384)=55.46$, $p<0.0001$) and a significant Group × Trial interaction ($F(43,384)=1.58$, $p<0.05$) suggesting that the groups improved during the course of testing, but that some groups improved more than others as testing progressed.

Direct and circle swims. Analysis of the direct and circle swim data in Figure 4c by one-way ANOVA confirmed the impression that the Naive pCPA group and the Naive and Pretrained pCPA + PRO 20 groups were impaired ($F(8,71)=6.23$, $p<0.0001$; Naive pCPA *vs* Control group, $p<0.05$; Naive pCPA + PRO 20 and Pretrained pCPA + PRO 20 groups *vs* $p<0.0001$ and $p<0.01$, respectively).

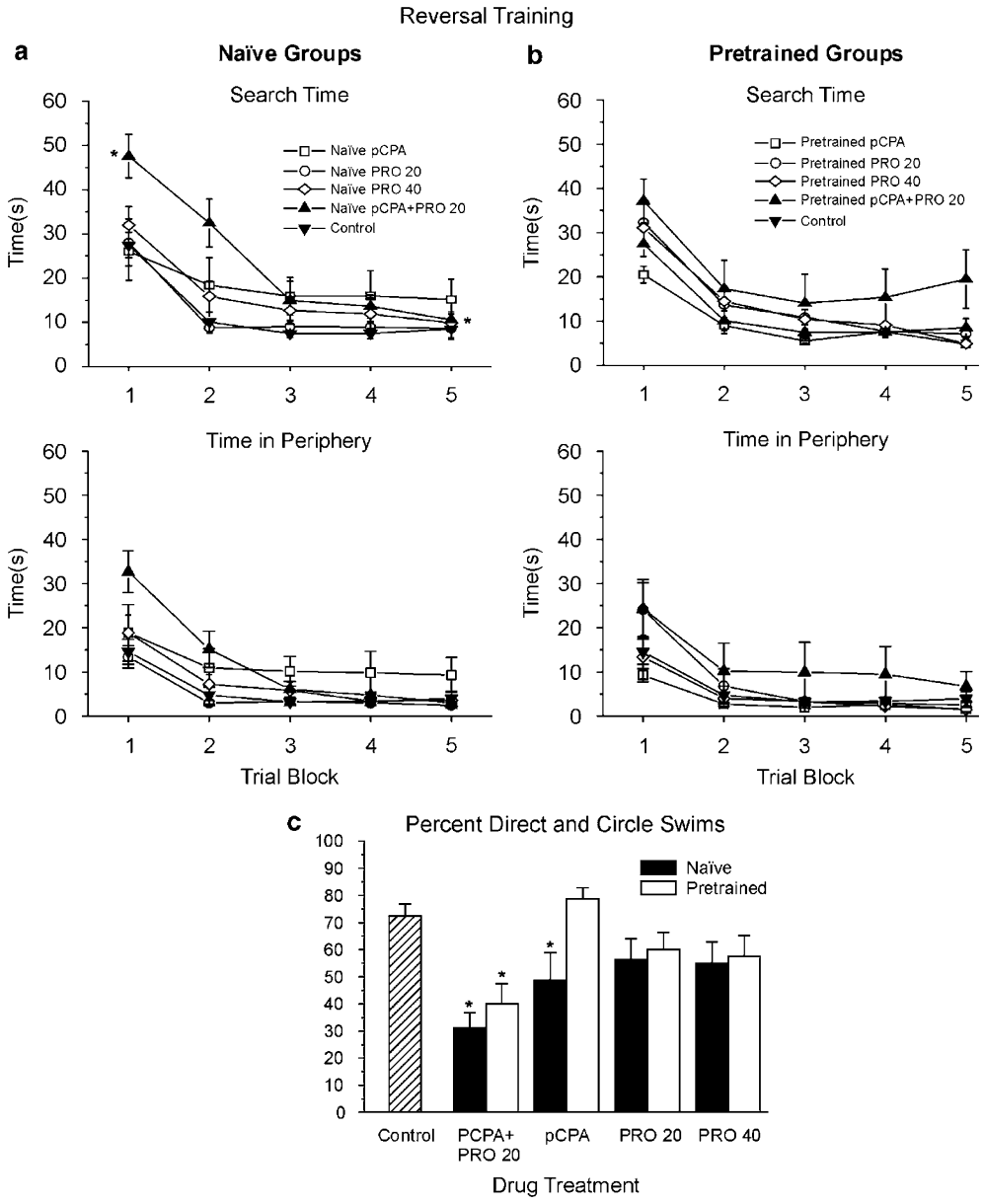

Figure 4 Experiment 2, reversal training. Hidden platform search time and time swum in the pool periphery by Naive groups (a) and by Pretrained groups (b); group mean percent direct and circle swims (c). The Naive pCPA + PRO 20 group was impaired on the search time measure, and the Naive and Pretrained pCPA + PRO 20 groups and the Naive pCPA group were impaired on the direct and circle swim measure. *Indicates values significantly different from controls, $p<0.05$ or better (see text). pCPA = *p*-chlorophenylalanine; PRO 20 = propranolol 20 mg/kg.

WM strategies. As shown in Figure 4a and b, the groups did not differ in periphery time ($p>0.05$), but there was a significant main effect of Trial ($F(2,162)=45.71$, $p<0.0001$), suggesting that the groups improved during the course of testing.

Beam Task

As was expected from previous research (Cain *et al*, 1996; Saucier *et al*, 1996), naive and pretrained rats that received the same drug treatment performed similarly on both measures of beam task performance ($p>0.05$). Therefore, Naive and Pretrained groups that received the same treatment were combined for analysis. Group mean time data to traverse the beam are shown in Figure 5a and group mean slips and falls data in Figure 5b. A one-way ANOVA indicated that the groups differed in time to traverse the beam ($F(3,60)=5.34$, $p<0.01$). *Post hoc* tests indicated that the pCPA + PRO 20 and PRO 20 groups took longer than Control group to traverse the beam. A one-way ANOVA indicated that the groups differed in number of slips and falls ($F(3,60)=5.71$, $p<0.01$), and *post hoc* tests indicated that the pCPA + PRO 20 group had more slips and falls than Controls group.

Correlations between Behavioral Measures

To examine relations between WM acquisition measures during spatial training and sensorimotor impairment measured with the beam task, product moment correlations

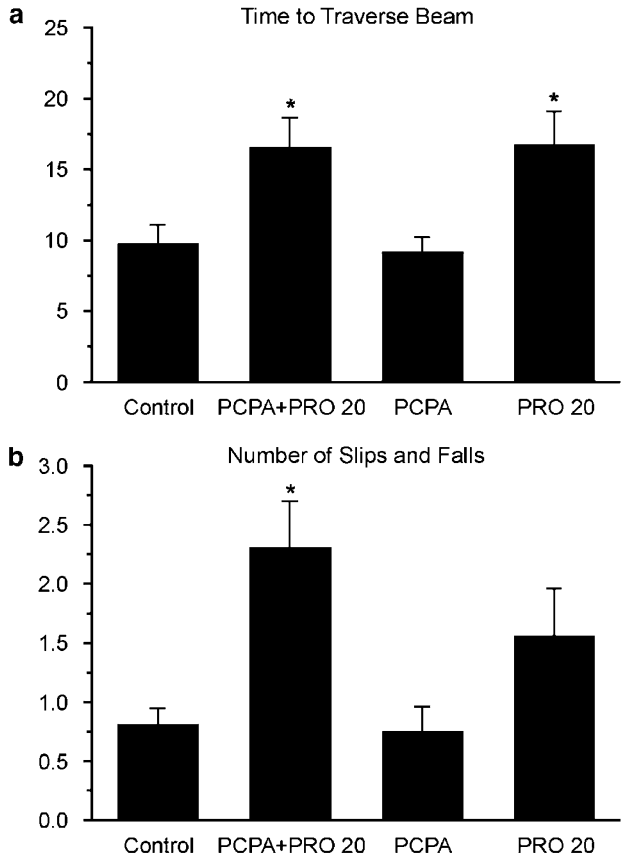

Figure 5 Experiment 2, beam task. Time to traverse the beam (a), and number of slips and falls (b). The pCPA + PRO 20 and PRO 20 groups were impaired on one or both beam task measures. *Indicates values significantly different from controls, $p < 0.05$ or better (see text). pCPA = *p*-chlorophenylalanine; PRO 20 = propranolol 20 mg/kg.

Table 2 Correlations between Spatial Training and Beam Task Measures

	Spatial training measures		
	Search time	**Direct and circle swims**	**Periphery time**
Beam task measures			
Traverse time	+0.28*	−0.29*	+0.20
Slips and falls	+0.32*	−0.20	+0.27*

Values represent product moment correlation coefficients between the behavioral measures indicated; data from all rats were used in the calculations. *$p < 0.05$.

were calculated between each of search time, percent direct and circle swims, and periphery swim time; and each of: beam traverse time, and slips and falls. Results are provided in Table 2. The absolute values of the correlation coefficients were small, but in each case the direction of the coefficient was consistent with a similar effect of treatments on both WM performance and beam task performance. For example, longer WM search time was associated with longer traverse time and more slips and falls in the beam task. Similarly, longer traverse time and more slips and falls in the beam task were associated with fewer direct and circle swims in the WM task. Four of the six coefficients were statistically significant at the $p < 0.05$ level (see Table 2). Although these coefficients account for a small amount of the variance, they suggest that a small portion of the treatment effect on WM performance during spatial training may have been due to drug-induced sensorimotor impairments.

DISCUSSION

The main finding of Experiment 2 was that either pCPA or PRO given alone produced few or no impairments on WM performance, whereas combined administration of pCPA and PRO impaired the performance of both naive and pretrained rats during both spatial and reversal training, and also impaired the rats in the beam task. These results are consistent with the impairments found in the Naive pCPA + PRO 20 group with the visible platform task in Experiment 1. This is the first finding of a 'global' WM impairment with combined administration of two agents that, at the high doses that were given individually, produced few or no WM impairments.

Drugs Administered Individually

The only group that was impaired by a single drug treatment was the Naive pCPA group, which was impaired only on the direct and circle swim measure in the hidden platform task. In contrast, the Pretrained pCPA group was unimpaired on all measures. This difference in outcomes suggests that prior acquisition of the required WM strategies, or stress reduction as a result of the pretraining experience (Beiko *et al*, 2004), or both, allowed the Pretrained pCPA group to learn the location of the hidden platform as effectively as Controls group. These results are consistent with earlier WM research that included Pretrained groups. For example, when pretrained rats were given any of a variety of neurotransmitter antagonists or agonists individually, such as NMDA, serotonergic, β-adrenergic, or muscarinic cholinergic antagonists, or a GABA agonist, there was no impairment in spatial place memory (Beiko *et al*, 1997; Cain, 1997; Cain *et al*, 1996, 1997, 2000; Morris and Inglis, 2003; Saber and Cain, 2003; Saucier and Cain, 1995; Saucier *et al*, 1996). The fact that there was no impairment with either dose of PRO on either version of the WM task is also consistent with the repeated failure to find impairing effects with a wide range of doses of PRO on the WM or radial arm maze tasks (Beatty and Rush, 1983; Beiko *et al*, 1997; Decker *et al*, 1990; Harder *et al*, 1996; Hiraga and Iwasaki, 1984; Saber and Cain, 2003; Richter-Levin and Segal, 1989; Riekkinen *et al*, 1992; Vanderwolf, 1987). Taken together, the previous and present findings suggest that among the systems studied, there does not appear to be a crucial single system that is required for the spatial memory component of the conventional WM task. The data suggest instead that a number of neurotransmitter systems normally contribute to spatial learning, and that the nature of any impairment that is produced depends on the past history of the animals,

the specific actions of the drugs, and the doses that are administered.

Naive rats exhibit a strong stress response to testing in the WM (Beiko *et al*, 2004; Holscher, 1999). Given the impairing effects of stress in the WM task (de Quervain *et al*, 1998; Holscher, 1999), the reduction in this stress response that results from Morris' pretraining (Beiko *et al*, 2004; Morris, 1989) may be a factor in the performance difference seen between the Naive and Pretrained pCPA groups during spatial training. Further, depletion of serotonin by pCPA appeared to exacerbate the aversive response to WM testing in naive rats. Unlike rats in all other groups, the rats in the Naive pCPA group were strongly reactive to both handling and to being placed into water by resisting capture and vocalizing when first placed into water. Their initial behavior in water usually consisted of rapid water-treading movements followed by rapid swimming movements. This reaction is consistent with previous reports of increased reactivity to stimuli in rats given pCPA (Brody, 1970; Dringenberg *et al*, 1995) and it sometimes resulted in swim paths that failed to meet the strict criteria for either a direct or circle swim. Although previous WM studies have not reported impairments in naive rats given pCPA, these studies did not make use of the direct and circle swim measure of memory for the hidden platform location (Beiko *et al*, 1997; Harder *et al*, 1996; Richter-Levin and Segal, 1989; Riekkinen *et al*, 1992). Because swim paths are examined more closely with this measure than with other measures of spatial memory, behavioral changes due to hyper-reactivity to both handling and immersion in water may be more likely to impact the direct and circle swim measure used here than the other measures used in previous studies. The strong reaction to immersion in water by the Naive pCPA group, together with the excellent performance by the Pretrained pCPA group on all measures, suggests that the direct and circle swim impairment in the Naive pCPA group may have been due to hyper-reactivity to handling and immersion in water rather than to a specific spatial memory impairment. Based on this information, the data in Experiment 2 appear to be consistent with earlier studies reporting no specific spatial memory impairment in rats given either pCPA or PRO.

Drugs Administered in Combination

The fact that combined serotonergic depletion and β-adrenergic antagonism impaired performance on both the visible and hidden platform WM tasks as well as the beam task suggests that this combined treatment caused a 'global' impairment that retarded or prevented normal acquisition of WM strategies and impaired spatial place learning. These impairments were obtained by the combined administration of treatments that, given individually, caused little or no impairment on the same tasks. There appear to be only two other known combinations of neurotransmitter antagonists that produce a similar global WM impairment in both naive and pretrained rats: scopolamine combined with either depletion of serotonin or antagonism of β-adrenergic receptors (Beiko *et al*, 1997; Saber and Cain, 2003). However, unlike serotonergic or β-adrenergic antagonist treatments given individually, scopolamine given alone causes a global WM impairment in naive rats (Beiko *et al*, 1997; Cain *et al*, 2000; Whishaw and Tomie, 1987). Thus the global impairment seen in *both* naive and pretrained rats in the present study with combined serotonergic depletion and β-adrenergic antagonism is especially noteworthy.

The use of pretraining to separate WM strategies acquisition from spatial place memory, together with separate measures of WM strategies use and memory for the hidden platform position, allowed us to address the question whether combined treatment with pCPA and PRO specifically impaired spatial place memory. During spatial training, both the Naive pCPA + PRO 20 and Pretrained pCPA + PRO 20 groups were impaired in WM strategies as measured by the periphery swimming measure. Importantly, the Pretrained pCPA + PRO 20 group failed to use the WM strategies that it had successfully acquired during pretraining carried out in the absence of drug treatment. This is a rare occurrence in WM studies that involve pretrained rats (Beiko *et al*, 1997), and it emphasizes the severity of the impairment. Given the fact that acquisition and use of WM strategies is essential for acquiring information about the location of the hidden platform (Cain, 1998; Morris, 1989; Whishaw, 1989), it is not possible to conclude that combined serotonergic depletion and β-adrenergic antagonism specifically impaired the establishment or recall of memory for the hidden platform location. Rather, the data suggest that the combined treatment caused a global impairment in adaptive behavior.

The data from reversal training are more suggestive of a specific spatial memory impairment. During reversal training, the Pretrained pCPA + PRO 20 group was impaired in the direct and circle swim measure but was not impaired in either the search time or periphery swimming measure. This indicates that by the second day of training, the Pretrained pCPA + PRO 20 group had reacquired command of WM strategies but still failed to demonstrate normal memory for the hidden platform location. Although this could be interpreted as a specific spatial memory impairment, this conclusion must be tempered by the fact that combined administration of pCPA and PRO impaired the same rats on the beam task, and that some measures of performance on the WM and beam tasks were correlated. These data seem consistent with the possibility that sensorimotor impairment due to the combined drug treatment may have contributed to the direct and circle swim impairment. Although this does not rule out a specific spatial memory impairment, it provides an alternate explanation for the poor performance in the direct and circle swim measure.

Basis of the Impairments

The basis of the finding that combined administration of pCPA and PRO impaired the performance of both naive and pretrained rats during both spatial and reversal training, whereas separate administration of the same agents produced few or no impairments, is not known. An important consideration in interpreting these results may be the highly diffuse nature of both serotonergic and adrenergic projection systems, with the potential to influence the function of many brain areas. Serotonergic neurons of the brain stem raphe nucleus project fibers widely in the forebrain, including the neocortex, hippo-

campus, and many limbic structures (Dahlstrom and Fuxe, 1964). Consistent with this, the diffuse serotonergic system together with the similarly diffuse cholinergic system that originates in the basal forebrain (Saper, 1984; McKinney *et al*, 1983) are known to be crucial for maintaining normal electrical activation of the neocortex and hippocampus (Vanderwolf, 1988). Simultaneous disruption of both of these systems can completely abolish spontaneous forebrain activation and severely impair a large variety of adaptive behaviors, including performance in both visible platform and hidden platform versions of the WM task by both naive and pretrained rats (Beiko *et al*, 1997; Vanderwolf, 1987). Thus, combined antagonism of serotonergic and muscarinic cholinergic systems produces a general impairment of both cerebral function and adaptive behavior.

The diffuse noradrenergic projection system that originates in the locus coeruleus has also been proposed as a basis for control of brain activation and adaptive behavior, but the evidence for this is less compelling than the evidence for serotonin and cholinergic systems in this role. Although there is clear evidence for the importance of noradrenergic mechanisms in post-training memory consolidation (Cahill *et al*, 2000), destruction of the ascending locus coeruleus projection system had little or no effect on neocortical activation (Jones *et al*, 1977; Vanderwolf and Baker, 1996; Whishaw *et al*, 1978) and many studies have found no impairment in performance on the WM or radial arm maze tasks in rats treated with a noradrenergic antagonist or given a lesion of noradrenalin containing neurons in the locus coeruleus before behavioral training (Beatty and Rush, 1983; Beiko *et al*, 1997; Decker *et al*, 1990; Harder *et al*, 1996; Hiraga and Iwasaki, 1984; Richter-Levin and Segal, 1989; Riekkinen *et al*, 1992; Vanderwolf and Baker, 1996). The results of the present study show that only combined β-adrenergic antagonism and serotonergic depletion caused a clear impairment in both the visible platform and hidden platform WM tasks. This outcome indicates an important role for β-adrenergic and serotonergic systems together in adaptive behavior that was not revealed by studies of the role of these systems separately in cortical activation or behavior. Although serotonergic neurons are known to directly inhibit noradrenergic neurons in the locus coeruleus through a 5-$HT1_A$ receptor (Haddjeri *et al*, 1997; Kaehler *et al*, 1999), in the present study only combined depletion/antagonism of both systems produced cognitive impairment. Thus a basis for a specific interaction of β-adrenergic and serotonergic systems in producing adaptive behavior is not clear from the present data.

Implications for Alzheimer Disease

It has been stated that Alzheimer patients will require symptomatic pharmacological treatment at all stages of the illness for the indefinite future (Tariot and Federoff, 2003). However, the current use of anticholinesterase drugs to improve cholinergic function has not been fully effective for many patients, and debate continues about their cost-effectiveness (AD2000 Collaborative Group, 2004). This is probably because Alzheimer disease typically involves the vast loss of synaptic contacts in cortex and elsewhere that normally release a number of brain neurotransmitter and neuropeptide systems including cholinergic, serotonergic, β-adrenergic, somatostatinergic, and GABAergic systems (Chen *et al*, 2000; Davis *et al*, 1999; Francis *et al*, 1999; Reinikainen *et al*, 1990), and this loss of multiple, often interacting systems may be important for the severe cognitive and behavioral impairments in Alzheimer patients. If true, optimal pharmacological treatment will require agents directed at a number of different systems in combination. Success with this approach requires that it be grounded in research that relates the cognitive and behavioral impairments to the underlying neurotransmitter and neuropeptide impairments.

As discussed above, past research is consistent in showing that greater than additive cognitive and behavioral impairments result from muscarinic cholinergic antagonism combined with either serotonergic depletion or β-adrenergic antagonism. The present results contribute to this topic by showing that although serotonergic or β-adrenergic antagonists given singly cause few or no impairments, both antagonists given together cause marked impairments. Considering that other combinations of neuroactive drugs can cause specific spatial memory impairment but not global WM impairment as found with pairwise combinations of muscarinic cholinergic, serotonergic, and β-adrenergic antagonists (see Cain *et al*, 2000), this suggests that pharmacological therapies involving a combination of agents directed at these particular systems might be a fruitful approach for further development of pharmacotherapies for Alzheimer disease symptoms. This approach may be more successful in targeting AD cognitive and behavioral symptoms than any single agent, and has the potential for optimization by tailoring treatments to the specific needs of patients based on individual differences in brain impairments.

In conclusion, individual depletion of serotonin or antagonism of β-adrenergic receptors caused few or no impairments in visible platform or hidden platform versions of the WM task. In contrast, naive rats given both treatments in combination were impaired in the visible platform task, and both naive and pretrained rats were impaired in the hidden platform task and also displayed sensorimotor impairments. This is the first finding of a global WM impairment with combined administration of two agents that, at the high doses that were given individually, produced few or no WM impairments. The data imply that serotonergic and β-adrenergic systems normally interact in a manner that is important for adaptive behavior, and suggest that impairments in these systems found in Alzheimer patients may contribute to the cognitive and behavioral impairments seen in these patients.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for income received from my primary employer, no financial support or compensa-

tion has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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