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Cognitive Dysfunctions Induced by Scopolamine Are Reduced by Systemic or Intrahippocampal Mineralocorticoid Receptor Blockade

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SMYTHE, J. W., D. MURPHY, C. TIMOTHY, G. H. HASSAN GUL AND B. COSTALL. *Cognitive dysfunctions induced by scopolamine are reduced by systemic or intrahippocampal mineralocorticoid receptor blockade.* PHARMACOL BIOCHEM BEHAV **56**(4) 613–621, 1997.—Central cholinergic blockade with scopolamine (SCOP) produces profound cognitive impairments in human and animal subjects. We hypothesized that cognitive deficits induced by cholinergic blockade originate partly from its ability to enhance reactivity to the environment, an effect that would be ameliorated by prior mineralocorticoid receptor (MR) blockade, because MR antagonists reduce reactivity to novelty. In the present study, we investigated whether or not systemic or intrahippocampal infusions of the MR antagonist spironolactone (SPIRO) would affect SCOP-induced cognitive impairments in a water maze task. Adult male Lister hooded rats (350–450 g) served as subjects. In Experiment 1, rats were administered SPIRO (0 or 100 mg/kg IP) followed 10 min later by SCOP (0, 0.5, or 2.0 mg/kg IP; n = 10/group). In Experiment 2, groups of rats implanted with hippocampal cannulae received central infusions of SPIRO (50 ng/μl; 3 μl in total) 10 min prior to SCOP injection (2.0 mg/kg IP; n = 6/group). Behavioural testing started 15 min after SCOP administration and consisted of a simple water maze task in which animals were required to locate a submerged platform using spatial cues. The testing regime consisted of two phases: a) acquisition, and b) retention, 24 h later. Peripheral, but not central, injections of SPIRO enhanced water maze performance during acquisition in SCOP-treated rats, as shown by shorter latencies and shorter distances travelled to locate the hidden platform. Both peripheral and central SPIRO administration reduced the long-term retention deficits in performance in the SCOP-treated animals. These data are in general agreement with a growing body of research suggesting that corticosteroid hormones interact with central cholinergic systems to affect both physiological and behavioural responses. MR blockade may reduce an animal's reactivity to the environment and enable it to selectively filter out extraneous stimuli that it would otherwise react to, thus impairing performance. © 1997 Elsevier Science Inc.

Cognition Water maze Mineralocorticoid Scopolamine Rat Cholinergic Acquisition Retention Hippocampus Peripheral Spironolactone

COGNITION has been defined as the implicit representation of an environment within the conscious brain in the absence of external defining features of that environment per se; concomitantly, rules and relationships between objects in the representation are understood and maintained, and an animal is able to extrapolate from its present circumstances to predict events in future time or locations (58). Animal models of cognitive function have been met with some suspicion because underlying behaviours used to assess cognition may also reflect conditioning or stimulus–response (S-R) learning (39). There has been considerable interest within the last 15 years regarding the utility of spatial navigation tasks such as the Morris water maze (MWM) (45) to assess cognitive function, since an animal is required to learn a set of reference rules to locate the position of a hidden escape platform but must use distally located cues to orient itself to each test trial and maintain a

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cognitive representation of the relative space–time relationships of those cues regardless of the animal's present position.

One key brain structure involved in the control or mediation of spatial behaviour is the hippocampal formation (cornu ammonis, dentate gyrus, and subiculum; commonly referred to as the hippocampus (2). Numerous studies have reported profound and protracted impairments in spatial or visually guided positional navigation, including the MWM, following hippocampal damage (45,49). The involvement of the hippocampus in such behaviours must be seen in light of its role in the control of a variety of physiological and behavioural conditions, including endocrine control (22), motor behaviour (59), and anxiety/fear (53,54). It has been suggested that the hippocampus serves as a central nervous system pacemaker and arousal center, and that such a function explains the diversity of its disparate roles (15,26).

Empirical support for regarding the hippocampus as important for arousal originates, in part, from electrophysiological studies. Field activity recordings made from the stratum oriens and stratum moleculare have revealed two principal waveforms: a) theta, a rhythmic, sinusoidal wave with a frequency range of 3–10 Hz; and b) large, irregular activity (LIA) that cycles interchangeably with theta (9,10). Motor activation is invariably accompanied by theta activity, but automatic behaviours such as grooming or chewing are usually accompanied by LIA. An alert, immobile rat can be induced to produce theta activity if presented with an arousing stimulus such as predator odour or tail pinch, a response that is blocked by prior administration of muscarinic cholinergic receptor antagonists injected peripherally or directly into the hippocampus (9,52). Furthermore, endocrine studies have revealed that hypothalamic–pituitary–adrenal (HPA) axis activity is regulated, in part, by negative feedback mechanisms in the form of mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) located on hippocampal neurons (11,13,14,51). Stress-induced corticosterone (CORT) secretion is elevated following hippocampectomy (22,34), and neonatally handled rats, which express higher hippocampal GR densities in adulthood, secrete less CORT when challenged by a stressor (43). Interestingly, intrahippocampal infusions of scopolamine, a cholinergic antagonist, exacerbate stress-induced CORT secretion, suggesting that hippocampal cholinergic function is essential for HPA axis negative feedback (7).

There is a vast literature demonstrating an important role for cholinergic systems in the performance of any number of learning and memory tasks, including the MWM (4,6,19,28, 31). There are numerous examples in which cholinergic blockade impairs learning and memory performance, whereas, conversely, cholinergic facilitation has been shown to enhance such performance (40,42). The contention that acetylcholine (ACh) is an essential component of cognition can also be derived from clinical data, especially in regard to age-related disorders such as Alzheimer's disease, where there is a direct association between the atrophy of basal forebrain ACh neurons and reduction in general cognitive abilities (8,23). It is intriguing and speculative, but it is likely that some of the involvement of cholinergic transmission in these cognitive functions may derive from its more rudimentary role in the mediation of attention and arousal (26,38,60).

We have hypothesized that hippocampal cholinergic systems are integral for an animal to monitor and amend its behaviour. Thus, aversive situations elicit cholinergic activity (1,18,20,21,32,33,44,46), which is necessary for the animal to assess the magnitude of the required response and the success of its subsequent motor output. Similar ideas have been proposed by Gray (25), with the notion of the hippocampus as a comparator mechanism, and by Bland (9,10), with his contention that cholinergic theta activity signals the force or acceleration of motor output required by an animal. We reasoned that with reduced cholinergic activity, an animal would be unable to gauge the severity of an aversive environment and would display behavioural hyperresponsivity in a manner similar to that observed with HPA axis activity when CORT levels are elevated following cholinergic blockade (7,53). As support for this idea we have reported that scopolamine pretreatment elicits a potent anxiogenic effect in the black–white box test following both peripheral and intrahippocampal administration (54,55). We contend that in the absence of normal cholinergic function, otherwise innocuous stimuli are perceived as threatening and aversive environments become even more threatening to an animal.

These data combine to suggest that hippocampal cholinergic blockade actually impairs cognitive function, not only by affecting some central representation of the environment but also perhaps by distorting the relative importance of insignificant stimuli that the animal now perceives as threatening. We have recently reported that the MR antagonist spironolactone has anxiolytic properties when infused directly into the hippocampus (56), an effect that occurs rapidly, which is suggestive of a nongenomic response (35–37,41,61). Oitzl et al. (47,48) have reported that CORT-induced reactivity to novelty is blocked by intraventricular injections of the MR antagonist RU 28318. Given the putative relationship between hippocampal cholinergic function and HPA axis control (7), and our findings of scopolamine-induced anxiogenesis (54,55), we decided to investigate whether or not deficits in a MWM task induced by cholinergic disruption would be affected by prior administration of an MR antagonist administered systemically or infused directly into the hippocampus. We hypothesized that cognitive deficits induced by cholinergic blockade originate partly from its ability to enhance reactivity to the environment, an effect that should be ameliorated by prior MR blockade, since MR antagonists alter reactivity to novelty.

METHODS

Subjects

Adult male Lister hooded rats (350–450 g) bred at the University of Bradford, were used as experimental subjects. At weaning, rats were housed in groups of five or six in clear polycarbonate cages with stainless steel lids. Standard rat chow (Purina) and tap water were provided ad lib. The colony room was environmentally controlled, with an ambient temperature of 20–22°C and a relative humidity of approximately 65%. The rooms were kept on a normal light cycle with lights on at 0800 h and off at 2000 h. Cage maintenance was undertaken twice weekly, but never on the day of behavioural testing.

Surgery

For experiments involving central infusions of drugs, groups of rats were initially implanted with hippocampal cannulae positioned bilaterally. Briefly, individual rats were anaesthetized with sodium pentobarbital (60 mg/kg IP) and placed into a stereotaxic apparatus with the plane between bregma and lambda levelled horizontally. A midline incision in the scalp was made from the region behind the eyes to the posterior region of the head but avoiding any muscles of the neck. The periosteum was retracted to expose the bare skull surface and permit visualization of the bregma. Holes were

drilled bilaterally above the hippocampus at A-P −3.3 and M-L ±2.5 mm, according the atlas of Paxinos and Watson (50). The cannulae consisted of two parts: a) a 26-gauge, stainless steel guide tube with a threaded, plastic top; and b) an injection stylet of 32-gauge stainless steel that fit tightly in the guide tube and protruded 0.5 mm beyond the tip of the tube. The guide tube was lowered into the hippocampus (D-V −2.4 mm from dura) and cemented into place using dental acrylic anchored to four small jeweller's screws positioned around the hippocampal entry holes. Drugs were administered through the injection stylet, whose additional length meant that infusions were targeted at the level of the stratum moleculare. Rats were allowed at least 3 weeks to recover from surgery before being tested.

Apparatus

The water maze consisted of a circular pool approximately 150 cm in diameter and painted white. The water depth was kept at 30 cm and the temperature at 30°C; the water was coloured opaque using a latex solution to mask the location of the escape platform. The escape platform was 10 cm in diameter and submerged 3 cm below the surface of the water. A heavy black curtain surrounded the entire maze to reduce distractions to the animal from experimenter movements. The walls of the maze enclosure had visual cues positioned around the pool, consisting of poster boards with broad black and white lines drawn either horizontally or vertically. Hanging above the pool about 180 cm from the water surface was a video camera that was attached to a video tracking system and computer. The computer was programmed to record path lengths travelled and latencies to locate the platform for each trial.

Behavioural Testing

The testing regime consisted of two phases: a) acquisition, and b) retention tests. During the acquisition phase, following the appropriate drug treatments, rats were placed onto the submerged platform for 5 s in order to form associations between the position of the platform and the various visual cues around the pool. The platform was always kept in quadrant 3. The rat was then lowered into the pool, facing toward the wall in quadrant 1, 2, or 4 (chosen at random), and allowed to swim for a maximum of 60 s or until it had located the platform. If the rat was successful in locating the platform, it was permitted 5 s on the platform before being positioned in the water in another quadrant (again chosen at random). A total of eight trials were administered to each rat. Rats unable to locate the platform on any trial were always positioned on the platform for 5 s prior to any further trials. At the conclusion of testing, rats were dried under a heat lamp and returned to their home cages. The retention phase of testing occurred 24 h later. Rats were tested as above, but with no further drug treatment administered.

Drug Treatments

In Experiment 1, rats were administered spironolactone (SPIRO; 0 or 100 mg/kg IP) followed 10 min later by scopolamine (SCOP; 0, 0.5, or 2.0 mg/kg IP). The vehicle for SPIRO was 25% propylene glycol + 70% saline and 5% ethanol, SCOP was dissolved in physiological saline. Behavioural testing started 15 min after the SCOP injection. Group sample sizes were 10 rats per drug combination. Experiment 2, rats received central infusions of SPIRO (50 ng/μl; 3 μl in total infused over a 40-s period) bilaterally 10 min prior to SCOP administration (2.0 mg/kg IP). The doses of drugs and infusion volumes were chosen based on previous published studies using these or similar drugs (6,14,35,37,52,56). Behavioural testing again started 15 min following the second treatment. Sample sizes were six per group.

Drugs

SPIRO and SCOP were both obtained from the Sigma Chemical Co. (Poole, Dorset, UK) and were prepared fresh on the day of testing.

Histological Analysis

One week after testing, rats previously implanted with cannulae were overdosed with sodium pentobarbital and transcardially perfused with saline + 10% formalin. Brains were removed and stored in formalin for later histological verification of guide tube placements. Brains were sectioned on a freezing microtome, taking 60-μm sections, and inspected visually for track marks from the guide tubes.

Statistical Analysis

Latencies to locate the platform (s) and path lengths taken (cm) were assessed by a split-plot factorial analysis of variance (ANOVA) with pretreatment (vehicle or SPIRO) and test treatment (dose of SCOP) as orthogonal factors, and test trial as the repeated measures factor. To facilitate analysis, the test trials were blocked in groups of two (i.e., trials 1 and 2, 3 and 4, 5 and 6, 7 and 8). To control for violations of sphericity of the variance/covariance matrix, the degrees of freedom for the repeated measures factor are reported as N (overall sample number) − K (number of group combinations). This is commonly referred to as a Greenhouse–Geiser correction (29) and prevents the inflation of the degrees of freedom associated with repeated measures designs. *Post hoc* tests were made using Bonferroni corrected *t*-tests designed to maintain the experiment-wise alpha level at $p < 0.05$ (12).

Ethical Approval

This research was carried out in accordance with the Animals (Scientific Procedures) Act 1986, under project licence PPL 50/01197.

RESULTS

Experiment 1: Peripheral Spironolactone

Latency to locate the platform during acquisition. A three-way ANOVA on time to locate the platform during the day 1 acquisition phase revealed a main effect of pretreatment with SPIRO [$F(1, 54) = 8.94$, $p < 0.004$] and a main effect of post-treatment with SCOP [$F(2, 54) = 11.3$, $p < 0.001$]. ANOVA also demonstrated a significant interaction between pretreatment [vehicle (VEH) or SPIRO] and trial block [$F(3, 54) = 5.4$, $p < 0.001$]. These data are depicted graphically in Fig. 1A. Both VEH–VEH- and SPIRO–VEH-treated rats showed improved performance (lower latencies to locate the platform) over the four trial blocks. By trial block 4, these groups displayed latencies approximately 50% of those from trial block 1 ($p < 0.01$). While VEH–VEH-treated rats did not differ from the SPIRO-treated rats, there was a marked effect of SPIRO in the SCOP-treated rats. Overall, rats administered VEH–SCOP exhibited longer latencies to locate the platform com-

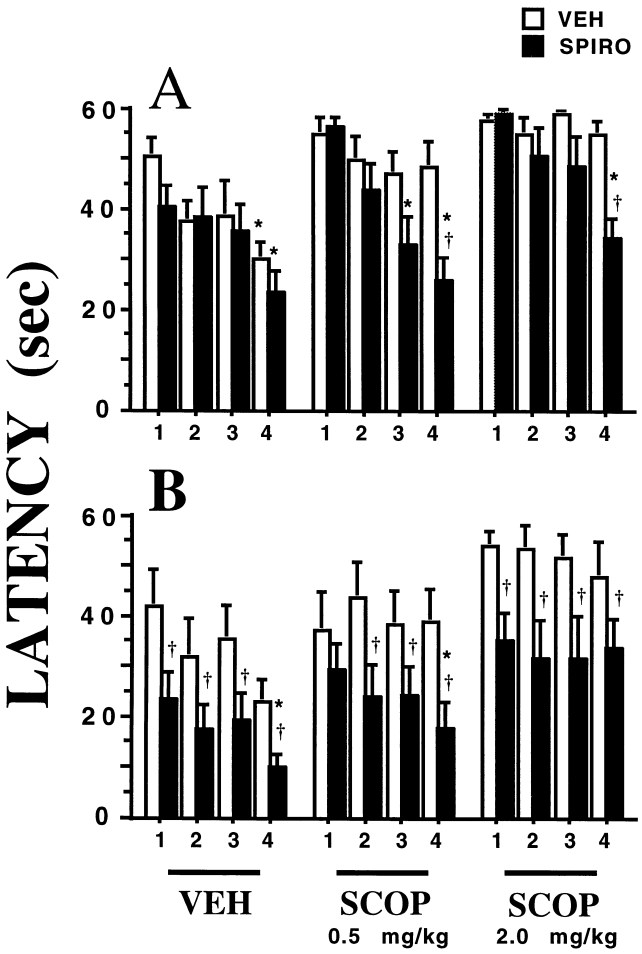

FIG. 1. Effects of peripheral injections of 100 mg/kg SPIRO on latencies to locate a submerged platform in VEH- and SCOP-treated rats. (A) Data obtained for the acquisition phase of testing. (B) Data obtained for the retention phase of testing performed 24 h later. Group means + SEM are shown. *Significantly different from the corresponding group in trial block 1 ($p < 0.05$–0.01). †Significantly different from the corresponding paired VEH or SCOP group in the same trial block ($p < 0.05$–0.01).

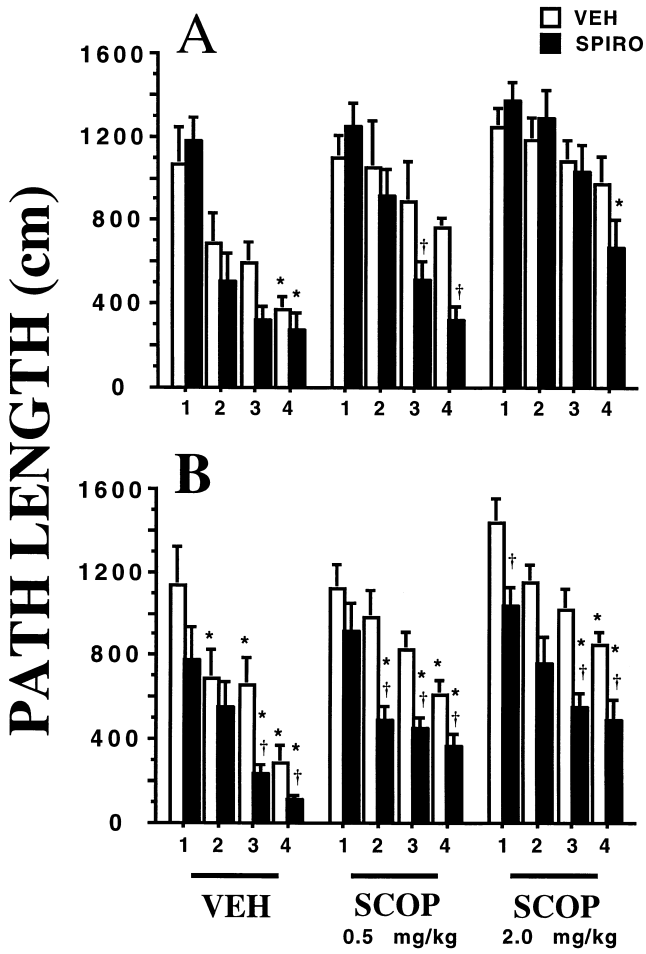

FIG. 2. The effects of peripheral injections of 100 mg/kg SPIRO on path lengths travelled to locate a submerged platform in VEH- and SCOP-treated rats. (A) Data obtained for the acquisition phase of testing. (B) Data obtained for the retention phase of testing performed 24 h later. Group means + SEM are shown. *Significantly different from the corresponding group in trial block 1 ($p < 0.05$–0.01). †Significantly different from the corresponding paired VEH or SCOP group in the same trial block ($p < 0.05$–0.01).

pared with VEH–VEH animals. Moreover, while VEH–VEH- and SPIRO–VEH-treated rats expressed equivalent learning and showed diminished latencies to locate the platform, SCOP pretreatment blocked learning. None of the SCOP-treated animals showed evidence of learning, as demonstrated by similar, high latencies to locate the platform over all four trial blocks. However, the deleterious effects of SCOP were reduced by prior administration of SPIRO. At the 0.5-mg/kg dose of SCOP, pretreatment with SPIRO produced learning curves over trial blocks 1–4 approximately the same as those observed in the VEH–VEH- and SPIRO–VEH-treated animals. By trial block 3, while the VEH–SCOP rats still exhibited poor performance, the SPIRO–SCOP-treated animals were significantly faster compared with those in trial block 1, and compared with the VEH–SCOP rats ($p < 0.01$). Furthermore, by trial block 4, the SPIRO–SCOP animals were not different from the VEH–VEH and SPIRO–VEH rats, but were considerably faster at finding the platform (twice as fast) compared with trial block 1 and VEH–SCOP rats in trial block 4 ($p <$ 0.01). Figure 1A also shows that the impairment in performance induced by the high dose of SCOP (2.0 mg/kg) was partially ameliorated by SPIRO pretreatment, although the effect was not evident until trial block 4. Presumably, the reversal of the SCOP-induced impairment depends on the severity of the deficit; SPIRO was capable of reinstating normal acquisition patterns, but the animals had to be trained for a longer time before this response was exhibited.

Path length travelled to locate the platform during acquisition. A three-way ANOVA on path length data revealed a pattern of results similar to those for latency to locate the platform data. While there was no main effect of pretreatment with vehicle or SPIRO [$F(1, 54) = 2.23$, NS], there was a significant interaction between trial block (1, 2, 3, or 4) and SPIRO administration [$F(3, 54) = 6.1$, $p < 0.001$]. There was again a main effect of SCOP treatment [$F(2, 54) = 12.5$, $p <$ 0.001] and a significant interaction between SCOP pretreatment and trial block [$F(6, 54) = 2.7$, $p < 0.05$]. These data are illustrated in Fig. 2A. Both VEH–VEH- and SPIRO–VEH-

treated animals showed progressively shorter path lengths over trial blocks 1–4, a finding that is in accordance with the shorter latencies to locate the platform, as described above. VEH–SCOP-treated rats displayed little improvement over trials, although there was a slight improvement by trial block 4 at the 0.5-mg/kg dose compared with trial block 1 ($p < 0.05$). There was no improvement in performance evident across trials at the highest dose of SCOP (2.0 mg/kg). Pretreatment with SPIRO of rats injected with SCOP produced a considerable improvement in performance. At the low dose of SCOP, rats given SPIRO beforehand displayed learning comparable to that of the VEH–VEH and SPIRO–VEH animals. By trial block 3, SPIRO–SCOP animals exhibited significantly shorter path lengths to locate the platform relative to trial block 1 ($p < 0.01$) and compared with the VEH–SPIRO animals in trial block 3 ($p < 0.01$). This reduction in the SCOP-induced impairment by SPIRO was even more pronounced by trial block 4 ($p < 0.01$ compared with trial block 1, and $p < 0.01$ compared with the VEH–SPIRO group in trial block 4).

Latency to locate the platform during retention. ANOVA on the data obtained for the retest 24 h after acquisition testing revealed a main effect of pretreatment with SPIRO [$F(1, 54) = 15.4$, $p < 0.001$] and a main effect of posttreatment with SCOP [$F(2, 54) = 5.5$, $p < 0.001$]. As shown in Fig. 1B, VEH–VEH-treated rats showed shorter latencies to locate the platform by trial block 4 compared with trial block 1 ($p < 0.05$). VEH–SCOP administration produced a dose-dependent impairment in latencies to locate the platform ($p < 0.05$–0.01 compared with the VEH–VEH group), and the animals displayed virtually no improvement in learning over trials. In sharp contrast to these data, SPIRO-treated rats exhibited improved performance regardless of whether or not they received VEH or SCOP. VEH–SPIRO-treated rats had latencies approximately 50% of those of the VEH–VEH animals ($p < 0.05$ at each trail block). While VEH–SCOP rats were impaired across trials 1–4, SPIRO treatment did reduce this impairment, although the reduction was less prominent at the 2.0-mg/kg dose compared with the 0.5-mg/kg dose of SCOP. At the 0.5-mg/kg dose of SCOP, SPIRO reduced latencies in trial blocks 2–4 ($p < 0.01$) to values comparable to those of the VEH–SPIRO group in the same trial blocks. The reduction in the SCOP impairment was less obvious at the 2.0-mg/kg dose of SCOP but was significant across trial blocks 1–4 ($p < 0.05$–0.01; VEH–SCOP compared with SPIRO–SCOP groups). There was, however, no improvement over trials 1–4 in the SPIRO–SCOP rats, as the improved performance remained at the same level across trials.

Path length travelled to locate the platform during retention. ANOVA on these data provided consistent results similar to those for the latency variable. There was a main effect of pretreatment with SPIRO [$F(1, 54) = 28.1$, $p < 0.001$] and a main effect of posttreatment with SCOP [$F(2, 54) = 10.5$, $p < 0.001$]. Inspection of Fig. 2B reveals that SPIRO administration augmented performance in all groups. VEH–VEH rats showed progressively improved performance (lower path lengths taken), an effect that reached significance on each trial block ($p < 0.005$–0.01 compared with trial block 1). However, this effect was enhanced by pretreatment with SPIRO in trial block 3 ($p < 0.05$) and trial block 4 ($p < 0.05$). VEH–SCOP-treated rats showed impaired performance compared with the VEH–VEH animals, an effect that was most prominent in trial block 4 ($p < 0.05$ for the 0.5-mg/kg dose, and $p < 0.01$ compared with the 2.0-mg/kg dose). However, SPIRO–SCOP-treated rats showed enhanced performance in trials 2 and 3 compared with the VEH–SCOP (0.5 mg/kg) animals ($p < 0.05$) and in trials 1, 3, and 4 compared with the high dose of VEH–SCOP ($p < 0.05$). These results largely mirror those obtained for the latency measures reported above.

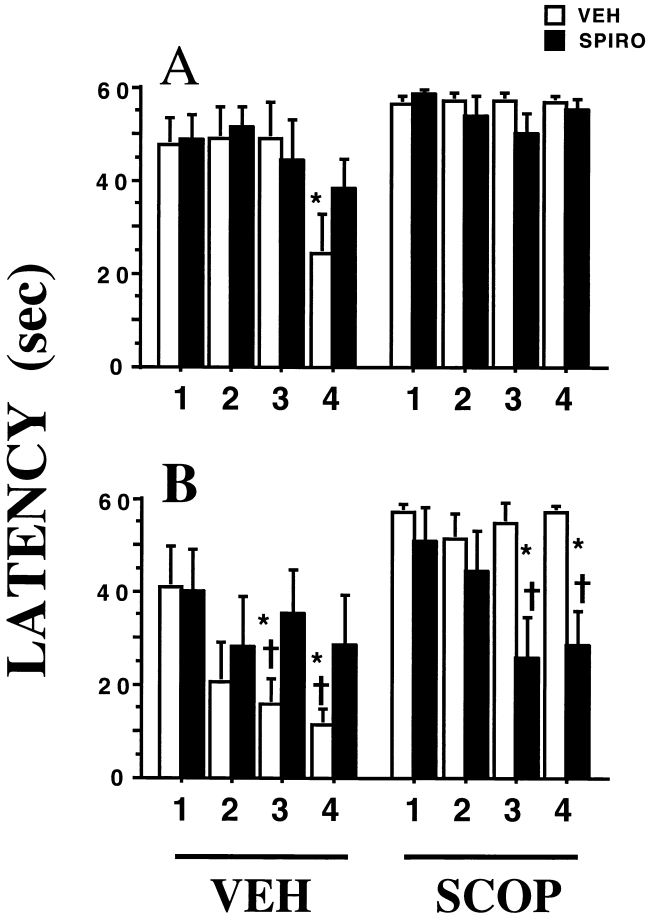

FIG. 3. Effects of intrahippocampal infusions of 150 ng of SPIRO on latencies to locate a submerged platform in VEH- and SCOP-treated rats. (A) Data obtained for the acquisition phase of testing. (B) Data obtained for the retention phase of testing performed 24 h later. Group means + SEM are shown. *Significantly different from the corresponding group in trial block 1 ($p < 0.05$–0.01). †Significantly different from the corresponding paired VEH or SCOP group in the same trial block ($p < 0.05$–0.01).

Experiment 2: Intrahippocampal Spironolactone

Latency to locate the platform during acquisition. ANOVA on latency to locate the platform following intrahippocampal infusions of SPIRO and peripheral injections of SCOP revealed a main effect of SCOP administration [$F(1, 20) = 13.0$, $p < 0.002$] but no effect of SPIRO treatment [$F(1, 20) = 0.6$, ns]. As shown in Fig. 3A, VEH–VEH-treated rats showed reduced latencies to locate the platform by the fourth trial block compared with the first trial block ($p < 0.02$). VEH–SCOP-treated animals exhibited no reduction in latencies over trial blocks 1–4; by trial block 4 their performance was unchanged from trial block 1, but latencies were significantly higher than those of the VEH–VEH rats in trial block 4 ($p < 0.01$). Unlike the peripheral injections of SPIRO, central infusions were without effect on latencies in either the VEH-

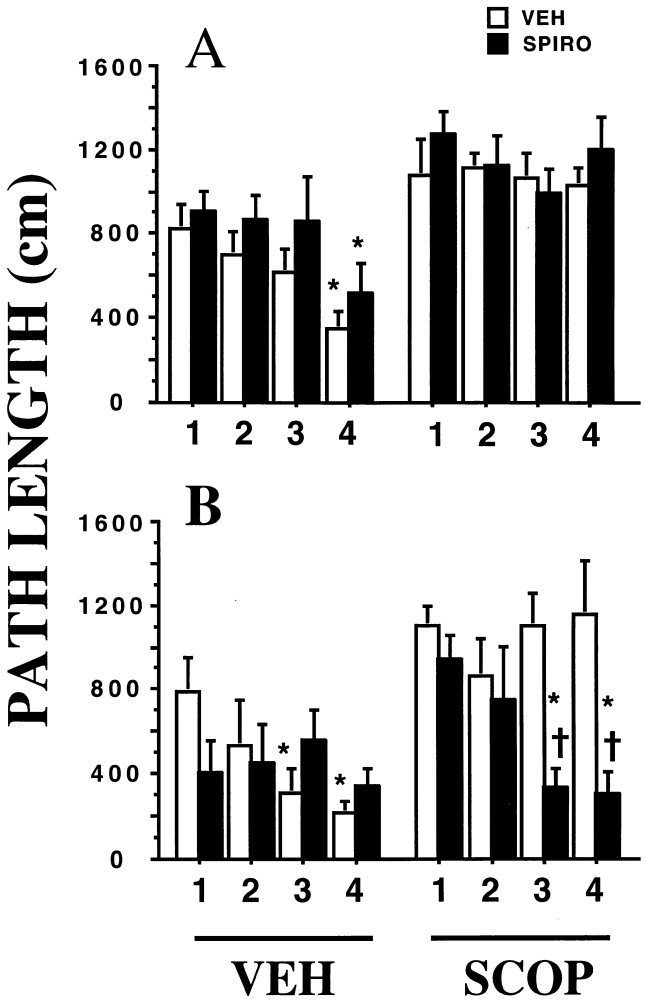

FIG. 4. Effects of intrahippocampal infusions of 150 ng of SPIRO on path lengths travelled to locate a submerged platform in VEH- and SCOP-treated rats. (A) Data obtained for the acquisition phase of testing. (B) Data obtained for the retention phase of testing performed 24 h later. Group means + SEM are shown. *Significantly different from the corresponding group in trial block 1 ($p < 0.05$–0.01). †Significantly different from the corresponding paired VEH or SCOP group in the same trial block ($p < 0.05$–0.01).

or SCOP-treated animals. In fact, VEH–SPIRO rats failed to show any improvement over trial blocks 1–4 and, in fact, appeared impaired in trial block 4 compared with the VEH–VEH rats, although the difference was not significant ($p < 0.13$).

Path length travelled to locate the platform during acquisition. ANOVA on the path length data revealed a pattern similar to that obtained for the latency data. There was again no significant effect of SPIRO pretreatment on path lengths [$F(1, 20) = 2.1$, NS], but there was a significant effect of posttreatment with SCOP [$F(1, 20) = 23.9$, $p < 0.001$]. As illustrated in Fig. 4A, VEH–VEH-treated animals showed a progressive improvement in performance (shorter path lengths travelled) that was significant when comparing trial block 4 with trial block 1 ($p < 0.02$). SCOP-treated rats exhibited no improvement over the trial blocks and were significantly impaired compared with the VEH–VEH rats in trial block 4 ($p < 0.05$). There was again no obvious effect of SPIRO on performance, although the SPIRO–VEH rats showed marginal improvement in performance in trial block 4 compared with trial block 1. The VEH–VEH and SPIRO–VEH groups did not differ in trial block 4. Furthermore, SPIRO pretreatment had no influence on the SCOP-induced behavioural impairment.

Latency to locate the platform during retention. ANOVA on the latency measures obtained in the retest 24 h afteer initial testing revealed no effect of pretreatment with SPIRO [$F(1, 20) = 0.4$, NS], but there was a main effect of posttreatment with SCOP [$F(1, 20) = 11.5$, $p < 0.001$]. The analysis also revealed a significant three-way interaction between pretreatment (VEH or SPIRO) × posttreatment (VEH or SCOP) × trial block (1, 2, 3, or 4) with [$F(3, 20) = 3.3$, $p < 0.05$]. Figure 3B shows means and SEM in graphic form. VEH–VEH animals exhibited a rapid and progressive improvement in performance over the trial blocks such that by the third and fourth trial blocks, latencies were significantly lower compared with trial block 1 ($p < 0.05$–0.01). No such improvement was evident in the SPIRO–VEH animals, who exhibited higher latencies compared with the VEH–VEH rats in trial blocks 3 and 4 ($p < 0.05$). Rats previously administered SCOP were still profoundly impaired compared with the VEH–VEH rats in trial blocks 2–4 ($p < 0.01$). However, while SPIRO–VEH rats displayed little evidence of improvement over trials, SPIRO–SCOP-treated animals showed a marked improvement (lower latencies) in trial blocks 3 and 4 compared with VEH–SCOP animals in trial blocks 1 ($p < 0.01$).

Path length travelled to locate the platform during retention. Analysis of path length data revealed main effects of both pretreatment with SPIRO [$F(1, 20) = 5.1$, $p < 0.03$] and posttreatment with SCOP [$F(1, 20) = 11.2$, $p < 0.003$]. ANOVA also demonstrated a significant three-way interaction between pretreatment (VEH or SCOP) × posttreatment (VEH or SCOP) × trial block (1, 2, 3, or 4) [$F(3, 20) = 6.0$, $p < 0.002$]. Group means ± SEM are illustrated in Fig. 4B. VEH–VEH rats took progressively shorter path lengths to locate the platform, an effect that was significant in trial blocks 3 and 4 compared with trial block 1 ($p < 0.01$). Rats that had received VEH–SCOP on the preceding test day showed no improvement over trials, and swam significantly longer distances in trial blocks 2–4 compared with the same trial blocks for the VEH–VEH rats ($p < 0.01$). Again, while SPIRO–VEH treatment led to minimal effects on performance, with the animals appearing to be somewhat impaired relative to the VEH–VEH rats, the combination of SPIRO–SCOP proved to be very effective at reducing distances swum. SPIRO–SCOP animals showed performance equal to that of the VEH–VEH and SPIRO–VEH rats in trial blocks 3 and 4, and profoundly reduced distances travelled compared with the VEH–SCOP rats in trial blocks 3 and 4 ($p < 0.001$). Thus, SPIRO very effectively blocked the enduring increase in path lengths caused by prior administration of SCOP.

Histological analysis. Reconstruction of serial brain sections revealed that guide cannulae placements for all rats were slightly dorsal to the stratum moleculare. While there were no obvious indications of the actual placements of the stylets, the positions of the guide cannulae, taking account of the additional 0.5-mm length of the stylet, confirmed that central infusions did target the region of the stratum moleculare of the dorsal hippocampus.

DISCUSSION

Cholinergic dysfunction causes profound cognitive impairments in a variety of tasks (6,19,57), and the results of the

present study confirm those findings. Following peripheral injections of the muscarinic antagonist SCOP, latencies to locate the platform and path lengths travelled to the platform in the MWM task were significantly elevated in a dose-dependent fashion compared with VEH controls. Moreover, in the acquisition phase, whereas control animals exhibited improved performance over trials, taking shorter path lengths and less time to locate the platform by the fourth trial block, no such improvement was noted in the SCOP-treated rats. The effect of SCOP was powerful enough to extend to the following test day, the retention phase, where the deficit in the MWM task persisted. This could mean that cholinergic blockade was still effective, because these animals did not behave like the VEH–VEH rats during the acquisition phase. Furthermore, these deficits were not attributable to any obvious motoric disturbances, because swim speeds (cm/s) were largely the same for all groups. It is perhaps not surprising that animals failing to acquire or encode the task on day 1 would also be debilitated on day 2 compared with rats that had successfully acquired the task on day 1. However, the actions of SPIRO, by itself and as an ameliorative agent versus SCOP, point to an interesting dichotomy between cognitive function and overt behavioural performance. Peripheral injections of SPIRO augmented MWM performance over that of the VEH-only rats, albeit marginally in the day 1 acquisition phase, but markedly in the day 2 retention phase. The fact that this facilitatory effect of SPIRO was expressed principally 24 h after administration suggests that a corticosteroid-mediated genomic mechanism is affected (36,41,61): blockade of some CORT-dependent response at MR presumably enhances behavioural performance. Even more provocative results were those concerned with the retention phase data. Both latencies and path lengths to localize the platform were reduced by previous injection of SPIRO, in both the VEH- and the SCOP-treated animals. The performance of the SCOP-treated rats administered SPIRO was not unlike that of the control animals, and the best overall performance was in the SPIRO–VEH rats, who were even more capable of solving the MWM task compared with the VEH–VEH controls. Thus, while SPIRO enhanced performance on day 1 in the SCOP-treated rats, the augmentation of that performance cannot fully account for the improved abilities of these animals on day 2. Clearly there is a protracted action of SPIRO that enables the rats to perform more efficiently on the second test day.

The dichotomy between day 1 performance and that of the retention phase is even more striking when one considers the results from Experiment 2, where SPIRO was infused directly into the hippocampus. Here we observed no particular effect of SPIRO on the acquisition of the MWM task, but we did see augmented performance during the retention phase. Thus, SPIRO–SCOP rats were markedly impaired on day 1, and indistinguishable from the VEH–SCOP animals, but were much improved on day 2, while the VEH–SCOP rats were still incapable of performing. These data clearly point out that even in the absence of obvious ability, the animal has actually acquired the ability to perform or at least can quickly acquire the task on day 2. Therefore, poor performance on day 1 does not preclude good performance on day 2. It is interesting that there was a discrepancy between the effects of peripheral and intrahippocampal infusions of SPIRO on the acquisition phase of testing. There are a few obvious explanations that come to mind that might account for this disparity. a) It is possible that a peripheral injection of 100 mg/kg of SPIRO results in a higher brain concentration of the drug compared with 150-ng infusions directly into the hippocampus. Thus, with higher concentrations resulting from the peripheral treatments, a stronger, more pervasive response is obtained that affects both acquisition and retention behaviour. b) Peripheral injections may provide greater brain concentrations of the drug, which then are able to target and encompass more hippocampal tissue than is possible with single infusion sites. With the cannulae positioned only in the dorsal hippocampus, drug effects may be much more specific, whereas intraperitoneal administration may well target both dorsal and ventral hippocampal regions. c) The differences in response patterns may reflect some heterogeneity of the brain mechanisms that control MWM behaviour. Peripheral injections will inevitably affect extrahippocampal MR sites (37,51), which may contribute in some unique fashion to MWM behaviour.

The subtle differences between peripheral and central injections aside, it is evident that hippocampal cholinergic systems and corticosteroids interact to control cognitive behaviour. The fact that MR blockade was effective at reducing the SCOP-induced impairment is perhaps not surprising given the role of MR in the mediation of spatial behaviour (47) and the fact that MR blockade reduces CORT-mediated reactivity to novelty (48). It is evident that cholinergic systems are activated under stressful or arousing conditions (18,33,46), and there is support for the notion that corticosteroid hormones are important modulators of this response (24,32). Hesen and Joels (27) reported that carbachol-induced membrane depolarizations were augmented in hippocampal slices from adrenalectomized rats and that MR stimulation with aldosterone reduced this excitatory response. Contrary to this result, it has been hypothesized that, overall, MR stimulation increases hippocampal cellular excitability while GR stimulation reduces excitability, by lowering and raising firing thresholds, respectively (35,37). Moreover, another line of evidence also argues for a direct relationship between cholinergic function and corticosteroid hormone action. Azmitia et al. (3) reported that septal stimulation thresholds required to elicit hippocampal theta activity were elevated after adrenalectomy; in addition, we have recently found that MR blockade decreases spontaneous theta production in urethane-anaesthetized rats (who exhibit only cholinergic theta) and elevates hypothalamic stimulation intensities required to drive theta activity (unpubl. obs.). These data combine to suggest that MR blockade actually has some anticholinergic properties. We have yet to formulate an explanation for this effect of MR blockade on theta generation in light of the results from the present investigation, since these findings appear to be contradictory.

Research with a somewhat different orientation has also suggested an association between learning and corticosteroid action. Measures of synaptic plasticity, such as long-term potentiation and primed bursting are reduced under conditions of high CORT levels induced by pharmacological means or by chronic stress (5,17). Low levels of CORT, on the other hand, facilitate the formation of potentiated responses (16). This inverted-U relationship probably reflects the differential occupation and activation of MR/GR and the concomitant effects on cellular excitation discussed above. Interestingly, synaptic potentiation has been reported to be maximal when induced by tetanic stimulation at theta frequencies (17), and it is enhanced during rhythmic membrane potential oscillations elicited by carbachol (30). These data corroborate the findings that corticosteroid hormones interact with cholinergic function to affect both physiological mechanisms and behavioural expression related to cognitive function.

It is difficult to determine the precise reason why MR

blockade should affect cognitive function that has been disrupted by SCOP administration. MR blockade may reduce the animal's reactivity to the environment and enable it to selectively filter out extraneous stimuli that it would otherwise react to, thus impairing performance. Our previous research shows that SCOP can increase an animal's reactivity to both stressful situations (7,53) and behaviourally arousing environments (54,55). The combination of water maze stress coupled with cholinergic blockade may produce elevated anxiety and/or despair resulting in impaired cognitive performance. Thus, MR blockade might act to counter these responses to SCOP, and both augment cognitive performance on its own and diminish the impairment due to cholinergic dysfunction. Whishaw and Petrie (62) have suggested that cholinergic disruptions do not affect learning and memory at all, but may in fact impair strategy selection such that rats choose maladaptive responses when placed in the pool. For instance, rats will scramble and swim by the pool edge rather than traverse the open surface searching for the platform. Cholinergic blockade may elicit a perseverative response whereby rats persist in one of these activities rather than seeking a platform. Presumably this is why rats will so easily learn to swim to a visible platform compared with a hidden platform they have had only brief exposure to. Again if this were a valid explanation for the SCOP-induced impairment, MR blockade might increase exploration across the open surface of the pool, thereby increasing the probability of stumbling upon the hidden platform. Our recent report that MR blockade increases exploration in the black–white box test would support such a contention (56).

In conclusion, MR blockade with SPIRO effectively reduced deficits in cognition induced by cholinergic blockade. The rapid effect of SPIRO to reduce SCOP's impairment during the acquisition phase suggests that a nongenomic mechanism may be involved, although the reduction in the long-term disruption caused by SCOP may be due to a genomic action. These data are in general agreement with a growing body of research suggesting that corticosteroid hormones interact with central cholinergic systems to affect both physiological and behavioural responses.

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