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Memory Following Cholinergic (NBM) and Noradrenergic (DNAB) Lesions Made Singly or in Combination: Potentiation of Disruption by Scopolamine[1,2]

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SAHGAL, A., A. B. KEITH, S. LLOYD, J. M. KERWIN, E. K. PERRY AND J. A. EDWARDSON. *Memory following cholinergic (NBM) and noradrenergic (DNAB) lesions made singly or in combination: Potentiation of disruption by scopolamine.* PHARMACOL BIOCHEM BEHAV **37**(4) 597–605, 1990.—Groups of rats were trained on either delayed matching or nonmatching to position tasks, then divided into four subgroups and given the following bilateral lesions: (a) SHAM [vehicle injection into the nucleus basalis magnocellularis (NBM) and dorsal noradrenergic bundle (DNAB)], (b) DNAB (6-hydroxydopamine lesion of the DNAB, vehicle into the NBM), (c) NBM (quisqualic acid lesion of the NBM, vehicle into the DNAB) and (d) DUAL (neurotoxin lesions of both DNAB and NBM). Following postoperative recovery, the DUAL lesion subjects were slightly impaired, but by the seventh day of testing all groups were performing at similar levels. This strongly suggests that quisqualate lesions of the NBM are not sufficient to produce severe and lasting mnemonic disorders resembling those seen in Alzheimer's disease (AD). These data also indicate that the noradrenergic system may not be of critical importance with respect to cognition. It was reasoned that an additional anticholinergic treatment might exacerbate an underlying deficiency. All groups were injected, peripherally, with the cholinergic antagonist scopolamine (0–0.5 mg/kg). This drug dose-dependently disrupted performance in all groups. Moreover, the highest dose had a marked effect in the DUAL group, impairing performance even when no mnemonic burden was present (at zero delay). The results suggest that cholinergic NBM and noradrenergic DNAB lesions produce only transient mnemonic deficiencies. A combination of the two can be disruptive, but longer term task (or reference) memory is the primary process affected, and only under certain conditions. The implication of these findings to research concerning animal models relating to Alzheimer's disease is discussed.

Memory Dorsal noradrenergic bundle Nucleus basalis magnocellularis Noradrenaline Acetylcholine Scopolamine Rat

THE past decade has witnessed a great deal of interest in the "cholinergic hypothesis of memory" (3, 9, 10, 21, 24), which proposes that the neurotransmitter acetylcholine has a pivotal role in the mediation of memory and other cognitive processes. This interest has been stimulated by research into the causes and possible treatment of Alzheimer's disease (AD), a severe and widespread form of dementia in the elderly. One of the outstanding characteristics of AD is a marked loss of cortical cholinergic activity originating from cell bodies in the basal forebrain, including the nucleus basalis of Meynert, coupled with mnemonic dysfunction (34). There is an extensive literature on the disruptive effects of cholinergic antagonists and lesions and, conversely, enhancing effects of cholinomimetics on cognitive performance in animals (1, 4, 11, 12, 21, 37, 48).

Evidence from studies such as these has led to the suggestion that cholinergic disruption, especially lesions of the nucleus basalis magnocellularis (NBM), the animal homologue of the nucleus of Meynert, may serve as a good model for the cognitive deficits seen in AD (16, 18, 25). However, there have been contradictory or confusing reports. Thus, ibotenic acid lesions of the NBM, which reduce cortical cholinergic (acetylcholine transferase, ChAT) activity by 17–50%, disrupt cognition in rats (13,15) and marmosets (38); however, the more specific quisqualic acid NBM lesions, where even greater reduction (>50%) of ChAT activity is

[1]Some of the data reported here were presented at the 3rd International Meeting of the European Behavioural Pharmacology Society (Noordwijkerhout, The Netherlands, 27 June–1 July 1990), and have appeared as an abstract (47).

[2]We humbly dedicate this paper to the memory of B. F. Skinner, whose immense influence on the behavioural methodology will be obvious.

seen, have fewer, if any, mnemonic effects (13,15).

With few exceptions, most of the lesion studies investigating mnemonic and cognitive processes have investigated selected pathways within the cholinergic system (for example, the NBM-cortical or septo-hippocampal projections). Moreover, both drug and lesion studies have concentrated on studying the effects of cholinergic manipulation alone; very few studies have considered the role played by other neurotransmitters also known to be deficient in AD (14). Indeed, evidence suggests that these neurotransmitters do not play a critical role in the disorder; for example, the reduction of dopamine beta hydroxylase, an enzyme which converts dopamine into noradrenaline, does not correlate with cognitive decline in AD (35). However, data from animal studies suggest that noncholinergic mechanisms may contribute to the cognitive disorders seen in dementia (7, 8, 54).

Although the majority of the available evidence suggests that monoamines do not have a major and direct role in cognitive processes such as learning and memory (20, 30, 36, 51), some authors have argued that noradrenaline (6, 39–41, 49) and serotonin (29,50) have important regulatory roles in behavioural processes. These intriguing hypotheses could help to explain why NBM lesions may be ineffective: a functioning noradrenergic/serotonergic regulatory mechanism could help overcome any deficiencies produced by the (NBM) lesions. It was these questions, relating to cholinergic and noradrenergic interactions, that we wished to address in this study.

EXPERIMENT 1

Using modified versions of Dunnett's (11,12) matching and nonmatching to position tasks, which are related to delayed response/alternation paradigms, we investigated the effects of (a) neurotoxin lesions of the dorsal noradrenergic bundle (DNAB), which projects from cell bodies in the locus ceruleus to cortex and hippocampal structures, (b) excitotoxin lesions of the nucleus basalis magnocellularis (NBM), which gives rise to the main cholinergic cortical projection, and (c) both areas lesioned together. In line with most previous reports, it was predicted that DNAB lesions would have little or no effect on performance, and that (quisqualate) NBM lesions would have only minor, if any, effects. Both lesions together might, on the other hand, produce severe disruption.

METHOD

Subjects

Fifty-three male PVG hooded rats (Bantin and Kingman, Hull, UK), weighing 225–250 g, were individually housed under diurnal conditions (lights on 0700 to 1900 h). All animals were trained on either matching (MTP) or nonmatching (NMTP) to position (see the Procedures section). The final group allocations were SHAM: 5 MTP, 6 NMTP; DNAB: 5 MTP, 6 NMTP; NBM: 8 MTP, 7 NMTP; DUAL (DNAB + NBM): 7 MTP, 9 NMTP. They had free access to water, but were food deprived to 85% of their free-feeding weights. They were tested in a darkened room where white noise (70 dB) was continuously present. The subjects had been used in studies assessing the effects of acute, peripheral, injection of vasopressin and cholinomimetic drugs. None of these treatments has chronic effects, and the present experiments were undertaken several weeks later.

Apparatus

Ten rodent operant chambers ("Skinner boxes": Campden Instruments, London, UK), each fitted with two retractable levers placed 15 cm apart, a centrally located illuminated food pellet dispenser fitted with a clear, hinged, Perspex® magazine flap connected to a microswitch, house light and three stimulus lights (one top centre, the other two 3 cm above each lever), were connected to Acorn System III microcomputers (Acorn Computers, Cambridge, UK) running on ONLIBASIC® software.

The three stimulus and one house light bulbs in this equipment had been replaced by light emitting diodes to provide a lower, but adequate, level of illumination [see (46)]. The magazine illumination was provided by a 24-V, 2.9-W bulb.

Procedures

Dunnett (11), and subsequently Sahgal (43), have described a matching to position (MTP) task which enables memory processes to be determined accurately in rats. In this modified delayed response task the rat is required to remember the spatial position of one of two (retractable) levers over various delay intervals. Rats learned this task readily, and performance (as percent corrects) decreased in a monotonic manner with increasing delay. We have trained rats on either MTP or nonmatching to position (NMTP), which is similar to MTP, the essential difference being that the correct response is one to the lever which had *not* appeared as the sample.

Previous work has indicated that MTP and NMTP can produce contradictory results (46). In the present study, therefore, both MTP and NMTP variants of this task were used. The rats were "autoshaped" to respond to the two levers (42). They were then divided into two groups, one to undergo matching to position (MTP) training, the other nonmatching (NMTP). In the first stage, no delays intervened between sample and choice lever presentation. A trial consisted of the following sequence of events. After a 10-s intertrial interval, either the left or the right hand lever would emerge; the stimulus light above it was also illuminated to provide an extra cue. The rat had to respond to the lever within 20 s, upon which the lever would retract and the magazine tray illuminate. This limited hold was introduced to encourage responding within a reasonable period of time; we have found that rats usually respond within 2 s. Next, the subject had to approach the tray and, within 20 s, operate the magazine flap. Both levers then emerged, and the stimulus lights above them were also illuminated. Rats in the MTP group now had to respond, for a food pellet, to the lever which had appeared as the sample. Those in the NMTP group, however, had to respond to the other one. These choice responses also had to be made within 20 s. Incorrect responses, or a failure to respond, resulted in a time-out of 20 s, during which all lights in the chamber were switched off, and all levers retracted; these were the only occasions when the house light was off.

Delays between the sample and choice lever presentations were introduced once the rats had learned the basic task. Now magazine responses following the sample presentation were ineffective until the appropriate delay interval had lapsed; the first response after this resulted in the choice levers being presented, providing this response occurred within 20 s. At first, only very short (up to 2 s) delays were programmed, and the duration was progressively increased to the final values of 0, 4, 8, 16, 32 and 64 s used in this study. Ninety-six daily trials were scheduled for each subject, with equal numbers of left/right stimuli and each delay presented in different pseudo-random orders.

Surgery

All surgery was done under deep pentobarbital (45 mg/kg, in

traperitoneally: IP) anaesthesia, and the rats had been treated with pargyline hydrobromide (Sigma, Poole, UK: 50 mg/kg, IP) 30 min prior to surgery. A stereotaxic instrument (David Kopf Instruments, Tujunga, CA) was used, and infusions made by a Schuco infusion pump (Schuco International, London, UK) via a 30-gauge needle. The general health, food and water consumption of all animals was carefully monitored following surgery.

DNAB lesions. The 11 animals in this group received 2 μl bilateral infusions of 6-hydroxydopamine hydrobromide (6-OHDA: Sigma, Poole, UK), 2 μg (base)/μl in 0.9% saline containing 0.1% ascorbic acid. The injection coordinates were 6 mm caudal to bregma ±1 mm from midline and 5 mm below dura with the incisor bar set 2.4 mm below the interaural line. The infusions were made over 4 min, and the injection cannula was left in place a further 3 min to aid diffusion and prevent tracking by the neurotoxin on removal. All rats in this group received bilateral, vehicle only, injections into the NBM (see below) at the same time.

NBM lesions. The 15 rats in this group were given 1 μl bilateral infusions of 0.12 M quisqualic acid (QUIS: Sigma, Poole, UK) dissolved in 0.9% saline. The coordinates were 1 mm caudal to bregma ±3 mm from midline and 7.6 mm below dura with the incisor bar set 3.3 mm below the interaural line. To promote diffusion, the cannula was left in place for a further 3 min. These rats also received bilateral infusions of vehicle into the DNAB (see above) during this surgery.

DUAL lesions. Using appropriate neurotoxins, the 16 animals in this group were given bilateral lesions of *both* DNAB and NBM, as described above.

SHAM lesions. The 11 control subjects were given bilateral infusions of vehicle into DNAB and NBM, as described above.

Neurochemical Determinations

At the conclusion of the experiment all animals were killed by decapitation and the brains rapidly removed and dissected on ice. Tissue samples were taken from temporal neocortex (in the region of the rhinal sulcus) and hippocampus, bilaterally, as well as the hypothalamus. These samples were frozen over liquid nitrogen and then stored at −70°C.

These areas were chosen since the DNAB extensively innervates the cortex and hippocampus, and the NBM projects to cortical areas. On the other hand, the second major ascending noradrenergic pathway, the ventral bundle (VNAB), projects mainly to the hypothalamus. Thus, lesions of the DNAB should leave hypothalamic NA intact. These three areas should therefore provide a good indication as to the extent and locus of the DNAB and NBM lesions.

Noradrenaline (NA). Samples from the three areas were sonicated in 0.2 M perchloric acid containing 1 mM EDTA and 4 mM sodium metabisulphite. After centrifugation 20 μl aliquots were injected onto a Waters HPLC system (Millipore, Watford, UK) comprising a WISP 712 autosampler with refrigerated sample compartment, model 510 pump, model 460 electrochemical detector and a 15 cm Resolve C18 5 μ column, maintained at 35°C. The mobile phase consisted of 50 mM sodium acetate, 20 mM citric acid, 3.75 mM sodium-l-octane sulphonate, 1 mM di-n-butylamine, 0.135 mM sodium EDTA and 5% v/v methanol at pH 4.3. NA for standard solutions was purchased from Sigma, Poole, UK. All other HPLC chemicals were purchased from BDH, Poole, UK.

Choline acetyltransferase (ChAT). Samples of cortex were sonicated in 9 volumes of 0.32 M sucrose containing 0.5% Triton X-100. ChAT was then estimated on aliquots using the method of Fonnum (17). Protein was estimated using the Lowry et al. (27) procedure. ^{14}C-Acetyl-CoA was purchased from Amersham International, Amersham, UK. All other chemicals were purchased from Sigma, Poole, UK and BDH, Poole, UK.

Performance Measures and Analyses

The performance measures included percent correct responses, number of missed trials, two nonparametric measures of bias developed from the theory of signal detection (B″ and the Responsivity Index, RI) and the bias measure Index Y (I_y). These bias indices have been described in greater detail elsewhere (44). B″ and RI provide measures of *perceptual* and *response* bias respectively, and I_y contrasts accuracy between the two levers:

$$I_y = \frac{\text{(absolute value of left minus right lever corrects)}}{\text{(total number of corrects)}}$$

The data were transformed as appropriate (arcsin: percents, bias indices; square-root: misses) and analysed by parametric analysis of variance (ANOVA) including 3-factor (lesion, task, delay) mixed measures analysis (52), using a modified analysis of variance program for microcomputers (26). When the F-ratio was significant [$p \leq 0.05$, using Ogasawara's (33) subroutine for calculating exact probabilities], means were compared by appropriate a posteriori tests, including the Duncan range statistic (5). For clarity, ANOVA F-ratios giving p values >0.1 are not listed. Finally, the delay main effects are not discussed in detail, unless worthy of comment. These terms were, as in all our previous studies, almost always highly ($p << 0.001$) significant.

RESULTS AND DISCUSSION

Preoperative Testing

Data were collected immediately prior to surgery, and analysed after grouping the animals according to the lesions they were due to receive. None of the performance, bias and miss measures suggested any difference; in other words, the different groups were performing, preoperatively, at similar levels.

Neurochemical Results

NA depletions in the DNAB and DUAL groups were satisfactory in all animals, and indicated that the VNAB (hypothalamic projections) had been spared (Table 1). The ChAT depletions in the NBM and DUAL groups were also satisfactory (Table 2), and consistent with previous reports using QUIS. All animals were therefore included in the behavioural analyses.

Postoperative Testing

The animals were allowed to recover from surgery for at least 14 days. On the first day following this period, they were rehabituated to the test apparatus. Testing commenced on the following day, and continued for 6 days thereafter. Data were recorded daily, but only the first and last (seventh) day's analyses are reported here.

Percent Correct

The first test day's data indicated that the lesion groups' performance differed, $F(3,45) = 2.938$, $p = 0.042$; subsequent analysis confirmed that this was due to a difference between the DNAB and DUAL lesioned animals [$p < 0.05$; mean percent correct ± SEM (standard error of the mean): SHAM, 73.90 ± 2.66; DNAB,

TABLE 1

LISTING OF NORADRENALINE LEVELS (NA, ng/mg WET WEIGHT, ± SEM)

Group	N	CORT	%	HIPP	%	HYPO	%
SHAM	11	0.260 ± 0.025	(−)	0.265 ± 0.006	(−)	2.022 ± 0.132	(−)
DNAB	11	0.028 ± 0.004	(89)	0.022 ± 0.005	(92)	1.949 ± 0.173	(4)
NBM	15	0.237 ± 0.022	(9)	0.255 ± 0.014	(4)	2.008 ± 0.222	(1)
DUAL	16	0.024 ± 0.004	(91)	0.015 ± 0.003	(94)	1.824 ± 0.124	(10)

Percent depletion is given in parentheses.

CORT, temporal neocortex; HIPP, hippocampus; HYPO, hypothalamus. Other abbreviations as in text.

75.60 ± 2.55; NBM, 67.73 ± 2.10; DUAL, 66.44 ± 2.15]. The DNAB group was (nonsignificantly) better than SHAM, while the DUAL lesion rats had the worst performance. Both MTP and NMTP groups performed at similar levels ($F<1$; mean percent ± SEM: MTP, 69.71 ± 1.95; NMTP, 70.38 ± 1.41).

However, by the seventh (last) day, none of the groups differed from each other ($F<1$). Thus, the lesion-induced deficiencies proved to be transient, disappearing in a week. These results are shown in Fig. 1. Moreover, on the last test day, MTP group animals were performing reliably better than NMTP [$F(1,45) = 4.067$, $p = 0.047$; mean percent ± SEM: MTP, 80.21 ± 1.67; NMTP, 76.78 ± 1.57].

Misses

On the first day, the DUAL lesion group made more misses than the other groups, $F(3,45) = 6.760$, $p = 0.001$. However, the total number of misses was small (<11% of trials) and, by the last day of testing, there were no group differences: <2.5% of trials were recorded as misses.

Bias Measures

None of the three (B″, RI and I_y) bias measures altered as a result of the lesions (all F's<1; mean values, over both days ± SEM: B″, 0.34 ± 0.02; RI, 0.35 ± 0.01; I_y, 0.24 ± 0.01), nor the task. As expected, bias increased with delay.

These results suggest that neither noradrenergic DNAB nor cholinergic NBM lesions produce severe memory deficits in rats. While the DNAB lesion results are not surprising, several authors have reported similar data (30), the NBM lesion results indicate that this preparation may not be as good a model for the cognitive deficits seen in Alzheimer's disease as has been argued (16, 18, 25).

TABLE 2

LISTING OF ChAT ACTIVITY (nmol/h/mg PROTEIN, ± SEM)

Group	N	CORT	%
SHAM	11	39.837 ± 2.438	(−)
DNAB	11	49.573 ± 2.352	(+24)
NBM	15	19.711 ± 1.312	(51)
DUAL	16	20.829 ± 1.097	(48)

Percent reduction is given in parentheses but note that the DNAB group, where no reduction was expected, actually registered an increase (indicated by the "+" sign). Only tissue from temporal neocortex (CORT) was assayed. Abbreviations as in text.

One point deserves mention. While DNAB lesions deplete both cortical and hippocampal NA extensively (about 90%), NBM lesions using QUIS reduce ChAT marker activity in the cortex only, and to a lesser extent (about 50%). Despite this difference, DNAB lesions had even less effect on performance than NBM lesions (however, the effect did not reach significance). Although it was not intended, nor is it possible on practical grounds, to lesion noradrenergic and cholinergic neurones in the same brain areas, and to the same extent, the asymmetry inherent in the lesioning procedures should be borne in mind.

Even combined DNAB and NBM lesions failed to produce a lasting impairment, contrary to what we had expected based on our own unpublished work and other studies (7,8). Moreover, the lack of disruption was quite striking, since neither accuracy (percent corrects) nor bias was altered.

One possibility is that NBM lesions, even in combination with NA depletion, are not sufficient to cause severe mnemonic disruption. For example, enough cholinergic activity may remain, enabling the subject to cope with the demands of the matching to position tasks. Alternatively, other cholinergic systems, for example the intrinsic cortical cholinergic neurones (in the rat) and the septo-hippocampal pathway, may possess the capacity to substitute for an inefficient NBM-cortical mechanism. If this is the case, then an additional insult to the cholinergic systems may be necessary before selective disruption due to NBM lesions is seen. This hypothesis was tested in the following experiment.

EXPERIMENT 2

Aigner et al. (1) have reported that (ibotenic acid) lesions of the NBM do not affect recognition memory, a finding in agreement with the results of Experiment 1. These authors also made the interesting observation that ChAT activity appeared to be unaffected in the inferior temporal cortex following NBM lesions in primates. This area is known to be crucial for visual recognition memory (32,45). In other words, the NBM lesions may not have disrupted those cholinergic cortical loci most involved in solving these visual matching tasks. However, Aigner et al. also assessed performance when the cholinergic systems in their lesioned animals were further depressed by peripheral injection with the muscarinic antagonist scopolamine (SCOP). Although SCOP produced dose-dependent impairments in both control and lesioned subjects, the effect was greater in the experimental group.

In other words, it is possible that deficiencies in performance following NBM lesions may become apparent only when the system is under pressure, i.e., when residual cholinergic activity is suppressed, or the cognitive effort is high. Accordingly, we measured performance in our lesioned rats following peripheral injection of SCOP. It was predicted that SCOP would have disruptive effects in the following order: DUAL (most disrupted, since both

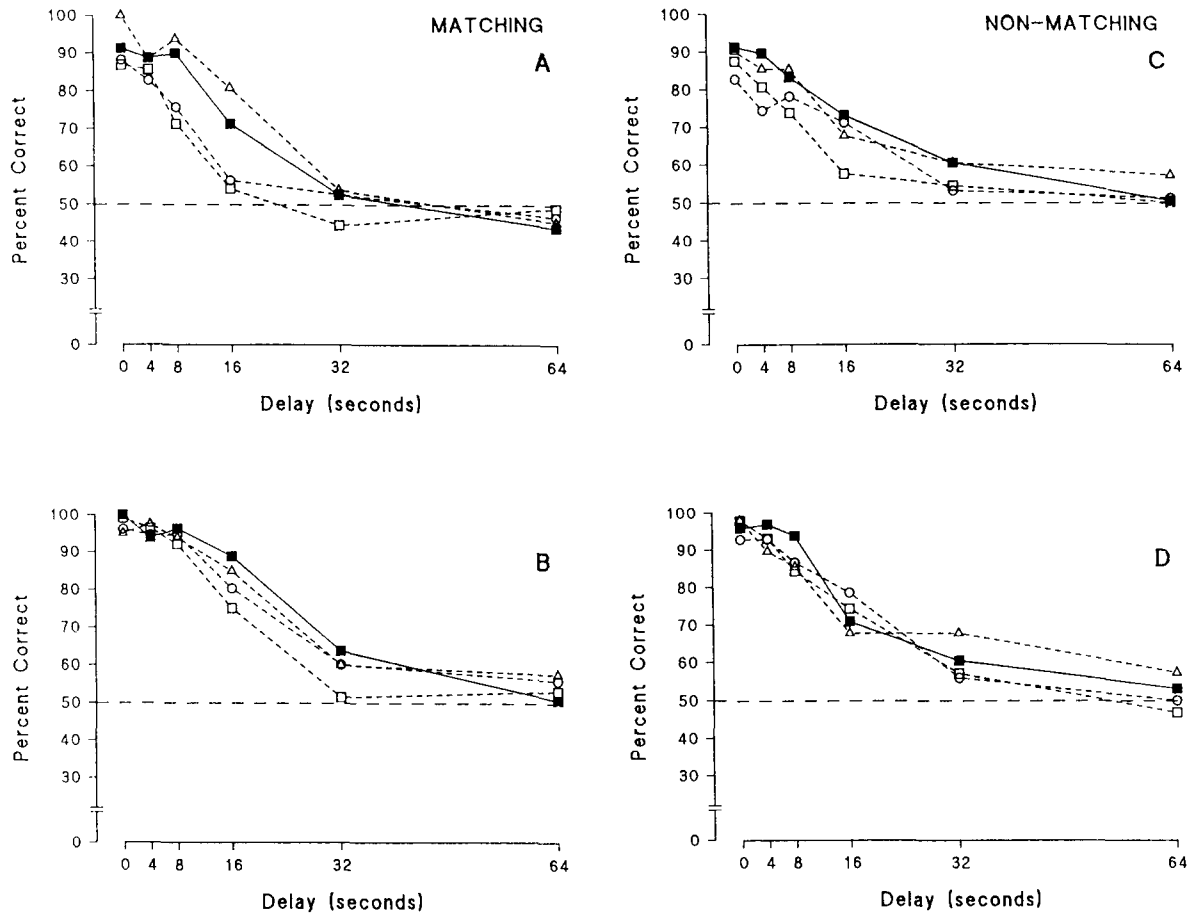

FIG. 1. Percent correct, Experiment 1. Top row (A, C): day 1 of testing; bottom row (B, D) day 7. The left column (A, B) refers to MTP, and the right column (C, D) to NMTP. Chance (50%) is indicated by the horizontal dashed line. ■, SHAM; △, DNAB; ○, NBM; □, DUAL; see text for lesion abbreviations.

cholinergic and NA-mediated regulatory processes were deficient) > NBM > DNAB ≥ SHAM (least affected).

METHOD

Subjects

Subjects were the same lesioned animals as in Experiment 1.

Apparatus

The same equipment was used as in Experiment 1.

Drugs and Test Protocol

In this experiment, subjects received 60 daily trials; all other conditions were as before. Saline injections (1 ml/kg) commenced several days before test data were collected. Scopolamine hydrochloride (SCOP) and the quaternary analogue methylscopolamine hydrochloride (MeSCOP), both obtained from Sigma, Poole, UK, were dissolved in 0.9% saline and injected, IP, 30 min prior to test. SCOP was given at the following doses: 0, 0.063, 0.125, 0.25 and 0.5 mg/kg. Once this sequence had been completed, all subjects were tested after 0 or 0.5 mg/kg MeSCOP, which does not cross the blood-brain barrier. At least two days lapsed between injections, during which the rats were not tested. Each rat received every dose of the drugs in an ascending sequence.

Performance Measures and Analyses

These factors were the same as before, but a 4-factor (lesion, task, dose, delay) mixed measures ANOVA was undertaken.

RESULTS AND DISCUSSION

MeSCOP Analyses

The behavioural data relating to this analogue are reported first, even though it was tested at the conclusion of the experiment. A significant change in behavioural performance would strongly indicate that the effect could have been mediated by peripheral, rather than central, actions. In other words, it is important to demonstrate that MeSCOP, injected at the highest SCOP dose (0.5 mg/kg) used in this study, did not produce the same changes as SCOP, on each of the indices measured.

None of the dose effects for percent corrects, or the three bias measures B″, RI or I_y were even remotely significant (all F's <1). We can therefore consider any changes following SCOP administration to reflect central action. However, the miss rate was increased, from about 1% (saline) to 6% (0.5 mg/kg), by

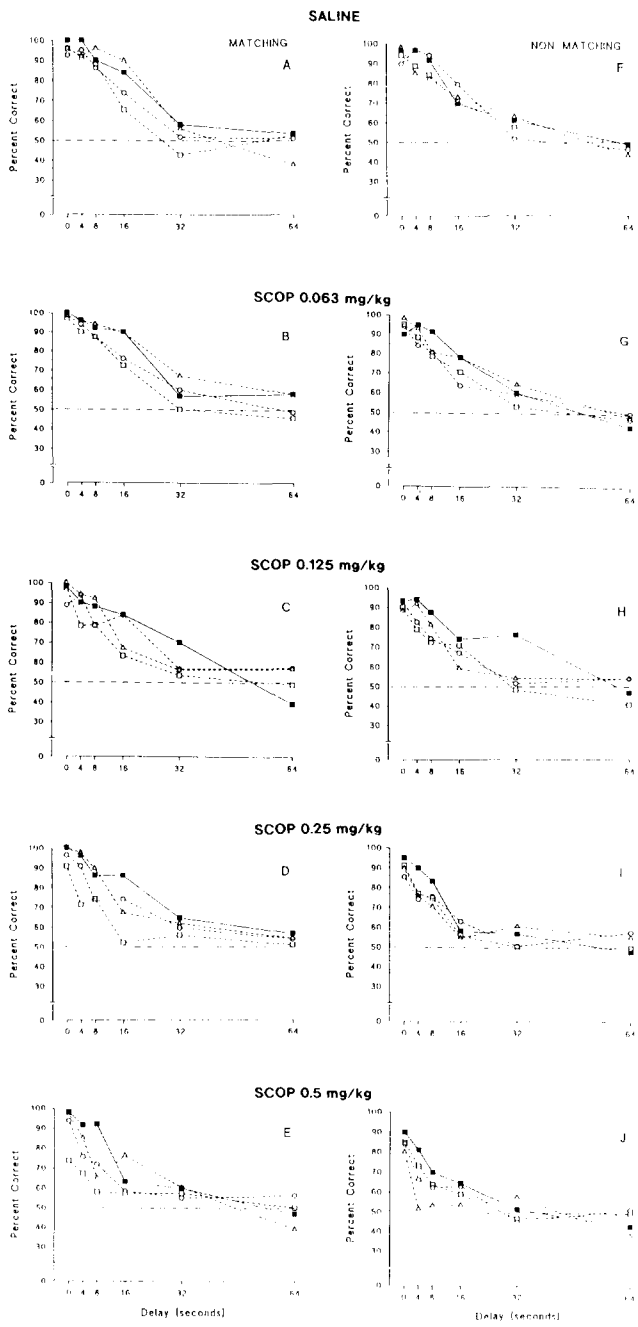

FIG. 2. Percent correct, Experiment 2. The rows, from top to bottom, refer to the different (0, 0.063, 0.125, 0.25 and 0.5 mg/kg) doses of SCOP, and the columns to MTP (left) and NMTP (right). See Fig. 1 legend and text for key to symbols and lesion groups.

MeSCOP, $F(1,45)=23.167$, $p<0.001$; interpretation of the data concerning this measure is therefore difficult.

SCOP Percent Corrects

All 4 main factors (lesion, task, dose and delay) were highly significant. The results are illustrated in Fig. 2, which shows percent corrects (vertical axes) versus delay (horizontal axes), with the lesion groups on each graph.

Lesion. In order of performance (mean percents ± SEM) the groups were: SHAM, 76.66 ± 1.25; DNAB, 73.11 ± 1.25; NBM, 72.27 ± 0.99; DUAL, 68.55 ± 1.00. Subsequent a posteriori analysis of the factor, $F(3,45)=4.731$, $p=0.006$, indicated, however, that only the DUAL group was significantly impaired compared to SHAM ($p<0.01$).

Task. The matching groups' percent correct performance was better, overall, than those in nonmatching [$F(1,45)=9.390$, $p=0.004$; MTP, 74.81 ± 0.82; NMTP, 70.24 ± 0.75].

Dose. SCOP produced a highly significant and dose-dependent disruption of performance, regardless of lesion, task and delay, $F(4,180)=31.420$, $p<<0.001$. A posteriori tests indicated that the saline scores (75.76 ± 1.24) did not differ from the lowest, 0.063 mg/kg, dose (76.30 ± 1.25), but that they differed from 0.125 mg/kg (73.12 ± 1.20; $p<0.05$) and the two higher doses (71.12 ± 1.20 and 64.87 ± 1.20 respectively; $p<0.01$ for both). In addition, the doses 0.063, 0.125, 0.25 and 0.5 mg/kg differed from each other ($p<0.01$), with the sole exception that 0.125 did not differ from 0.25 mg/kg.

Delay. As expected, performance declined monotonically with delay, $F(5,225)=274.099$, $p<<0.001$. Moreover, each delay was significantly ($p<0.01$) different from the adjacent one. The mean percent corrects (± SEM) for the 6 delays (0, 4, 8, 16, 32 and 64 s) were: 92.92 ± 0.68, 85.58 ± 1.03, 79.74 ± 1.10, 69.36 ± 1.10, 56.18 ± 0.95 and 49.62 ± 0.89 respectively.

In addition to these main factors, two (out of the eleven) interaction terms were also significant. These were the dose × delay, $F(20,900)=5.646$, $p<<0.001$, and lesion × dose × delay, $F(60,900)=1.677$, $p=0.002$, terms. The former reflects the fact that SCOP could not radically disrupt performance at the longer delays (due to a "floor" effect), but did so at the shorter delays, where performance was good. The latter term suggests that at least one group was differentially affected by particular doses at certain delays. From Fig. 2, we can see that the DUAL group's performance was generally worse, at higher (>0.063 mg/kg) doses of SCOP, than the others. A posteriori analyses indicated that this disruption was especially marked at the shorter (<16 s) delays.

SCOP Misses

The lesion factor was significant, $F(3,45)=3.072$, $p=0.036$, and both the DNAB (percent misses ± SEM: 6.8 ± 0.8, $p<0.01$) and DUAL (5.2 ± 0.5, $p<0.05$), but not NBM (4.7 ± 0.5), groups were making more misses than SHAM (2.0 ± 0.3).

However, although the overall number of misses recorded was low (even at the highest dose of SCOP, <12% of the trials were missed), the observation that MeSCOP also increased misses suggests that the disruption was confounded by peripheral actions.

SCOP Bias Measures

Neither the B″ ($F<1$) nor RI, $F(3,45)=2.325$, $p=0.086$, lesion effects were significant (mean absolute values ± SEM: B″, 0.41 ± 0.01; RI, 0.39 ± 0.01). However, the response bias RI depended on the task, $F(1,45)=7.041$, $p=0.011$: nonmatching was associated with greater bias (NMTP, 0.42 ± 0.01) than matching (MTP, 0.36 ± 0.01). Bias, as determined by both indices, increased very significantly with dose and delay.

Analysis of the significant, $F(3,45)=2.898$, $p=0.044$, I_y measure indicated that the DUAL group developed a bias towards making more correct responses on one of the levers ($p<0.01$). The mean values of the index were: SHAM, 0.23 ± 0.02; DNAB, 0.27 ± 0.02; NBM, 0.28 ± 0.01; DUAL, 0.34 ± 0.02. Moreover, nonmatching was associated with greater bias [$F(1,45)=4.334$, $p=0.041$: NMTP, 0.31 ± 0.01; MTP, 0.26 ± 0.01]. Like the other bias measures, I_y increased with both dose and delay.

These results support our prediction that the additional disruption posed by SCOP would affect performance in the order DUAL > NBM > DNAB > SHAM. Also, they confirm that NBM lesions, at least in rats, not only fail to produce permanent mnemonic deficiencies resembling those in Alzheimer's disease, but that even major loss of noradrenergic function, combined with NBM damage, may be ineffective. Impairments appear only when residual cholinergic activity is blocked by SCOP and the noradrenergic system is also impaired. Thus, in order to mimic the impairments seen in AD, more or less total "DUAL" (cholinergic plus noradrenergic) lesions may need to be made, with activity in non-NBM cholinergic mechanisms, known to be deficient in AD, also being reduced, perhaps by selective antagonist drugs or, more elegantly, by appropriate surgical lesions. The latter approach is more selective and would enable different cholinergic areas to be manipulated individually; peripherally injected drugs have a blanket effect on several cholinergic systems in the brain, including any lacking a noradrenergic input, and this creates some difficulties in interpreting the data.

The general lack of effect on B″ and RI indicates that the performance decrements were not caused by perceptual and response biases, respectively. The significant I_y result, however, suggests that the subjects were adopting a lever (position) preference type of strategy: the significant increase in this index suggests that the DUAL group rats tended to concentrate their (sample) responses on one particular lever, while ignoring the other one on at least some trials. This reduces the need to retain information concerning the "correct" lever and hence minimizes the cognitive effort required, but at the cost of some missed opportunities (on the ignored lever). Such "cognitive bias" strategies have been observed by us in earlier drug studies (43,44).

GENERAL DISCUSSION

In summary, lesions of the cholinergic and noradrenergic cortical pathways (NBM and DNAB) in rats do not produce the persistent memory impairments seen in patients suffering from Alzheimer's disease (AD). Even the combined lesions were ineffective and further cholinergic disruption, using the centrally acting muscarinic antagonist SCOP, was found to be necessary to produce reliable performance impairments.

An obvious comment would be that the lesions, particularly of the NBM, were incomplete. However, the neurochemical analyses indicate that (a) NA depletions were extensive, and in line with independent reports (30,40) and (b) QUIS-induced cholinergic lesions resulted in (marker activity) reductions to levels *below* that often seen following the more commonly used excitotoxin ibotenic acid which, moreover, produces marked deficits (13,15). Thus, insufficient depletions cannot account for our behavioural results. On the contrary, it is likely that ibotenate lesions are less selective, damaging other (catecholaminergic) systems in the region of the globus pallidus, and it may be this unintentional damage which produced the behavioural deficits reported earlier.

These results have obvious implications for current research concerned with the development of an appropriate animal model for AD. Early suggestions, based both on neurochemical and neuropathological data from human postmorten brain, and animal studies, that the NBM-lesioned rat might be a good model (16, 18, 25), are not supported. However, the results from this study support recent findings (13,15) that the NBM pathway may not be crucial to labile short-term memory processes ("working memory"), although a role for the NBM-cortical system in long-term procedural ("reference") memory cannot be excluded. It has been suggested (15) that the hippocampal pathway may be more important with respect to (working) memory processes.

Our data are also in agreement with reports (1) that NBM lesions in primates do not disrupt recognition memory and that an additional insult to the cholinergic system with SCOP might be necessary. Indeed, the present findings go one stage further in suggesting that noradrenergic damage is also required. Whether or not other neurotransmitter disruptions (for example dopamine, serotonin and the neuropeptides) would, in combination, produce even greater deficiencies remains to be seen.

Evidence from studies on aged animals and humans also suggests that the catecholamines might be involved in cognitive dysfunction. It has been shown (2) that clonidine, an α_2-adrenergic agonist, ameliorates age-related cognitive decline in monkeys, and a highly significant correlation ($r = .9776$, $N = 6$) between passive avoidance performance and locus ceruleus (the origin of the DNAB) cell counts in mice has been reported (54). Others (28) have argued that diminished catecholamine function is relevant to dementing disorders in humans.

Relatively few studies have considered the effects of combined noradrenergic-cholinergic lesions. There are preliminary reports (22) suggesting that the addition of a noradrenergic to a cortical cholinergic lesion affects the ability of physostigmine to normalize the memory deficit. Others (7,8) report that noradrenergic depletion potentiates the effects of cholinergic blockade in mice. Our results support these findings.

It has been argued (39,41) that the catecholamines are involved in behavioural arousal, and there is evidence (31) to suggest that cholinergic-noradrenergic interactions also play a role in this process. Gold and Zornetzer (19) have argued lucidly that manipulations of a wide range of neurotransmitters, including NA, can indirectly influence memory by actions upon arousal functions. Arousal is a crucial determinant of performance, affecting it in an "inverted-U" shaped manner (23,53). According to one model (39,41), the role of NA is to optimize behavioural performance by ensuring that arousal remains at a peak level. Because of the complexity of these arousal/performance mechanisms, noradrenergic malfunction might manifest itself *only* when the subject was expected to perform under (cognitive) pressure. This might help explain the results reported here. It is argued that these matching tasks might have been so (unavoidably) overtrained that, in Experiment 1, none of the subjects was under extreme pressure, and performance was unaffected. But when SCOP was injected (Experiment 2) this may no longer have been the case; the biggest decrement would be expected in the DUAL group, since both cholinergic (cognitive) and noradrenergic (arousal regulation) mechanisms were defective.

In conclusion, multiple neurotransmitter manipulations in animals, reflecting as they do the variety of neurochemical losses seen in dementia, might provide a more appropriate approach towards modelling the complex cognitive deficits seen in AD.

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