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Effects of *p*-Chlorophenylalanine and Methysergide on the Performance of a Working Memory Task

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JÄKÄLÄ, P., J. SIRVIÖ, P. RIEKKINEN, JR. AND P. RIEKKINEN, SR. *Effects of* p-*chlorophenylalanine and methysergide on the performance of a working memory task.* PHARMACOL BIOCHEM BEHAV **44**(2) 411–418, 1993. – The present study investigated the effects of serotonergic dysfunction on working memory. Therefore, the effects of inhibition of serotonin [5-hydroxytryptamine (5-HT)] synthesis induced by *p*-chlorophenylalanine (*p*-CPA) and pharmacological blockade of 5-HT receptors by methysergide on the performance of rats in a delayed nonmatching to position task assessing spatial working memory were studied. Methysergide (1.0, 5.0, or 15.0 mg/kg) significantly disrupted behavioral activity of rats and decreased the percent correct total responses. However, the impairment in the percent correct responses was delay independent, indicating a nonmnemonic disruption of the performance. *p*-CPA (500 mg/kg/day × 3) induced an almost total depletion (>97%) of frontal cortical and hippocampal serotonin and its major metabolite 5-hydroxyindoleacetic acid and slightly affected noradrenergic and dopaminergic systems. *p*-CPA treatment did not affect the percent correct responses. However, the behavioral activity of rats was slightly decreased by *p*-CPA. The disruptions in behavioral activity and the percent correct responses induced by methysergide (2.0 mg/kg) were not abolished by *p*-CPA. The present results do not support any important role for the serotonergic system in spatial working memory as assessed using the delayed nonmatching to position task.

Delayed nonmatching Working memory Serotonergic system *p*-Chlorophenylalanine Methysergide Rat

THE ascending chemically directed projections (cholinergic, dopaminergic, histaminergic, noradrenergic, and serotonergic projections) from the brain stem and basal forebrain widely innervate the forebrain, including the cortical mantle and limbic system (15). These subcortical ascending systems have been shown to have state-dependent effects on the function of information networks of the forebrain underlying higher cerebral functions, such as attention and vigilance, as well as learning and memory functions (25). Degeneration of these subcortical transmitter systems, including the serotonergic system (9,32,49), has been suggested to contribute to cognitive disorders related to aging, with this being accentuated in Alzheimer's disease (AD).

The brain pathology and cognitive symptoms in patients with AD are so severe at the time of death that adequate information for clinicopathologic correlations is difficult to obtain (5). To determine the functional consequences of specific pathologic changes in the ascending chemically directed subcortical transmitter systems observed in AD patients, systematic studies using rodents or subhuman primates as animal models of dementia are needed (5). The rationale for studying the possible relationship between the serotonergic neuropathology and AD-related cognitive deterioration in animal models has been to investigate the effects of lesions of the serotonergic system, brain serotonin [5-hydroxytryptamine (5-HT)] depletion, or pharmacological blockade of 5-HT receptors on higher cerebral functions (2). The most commonly used procedures to lower endogenous 5-HT levels throughout the brain are peripheral administration of the tryptophan hydroxylase blocker *p*-chlorophenylalanine (*p*-CPA), which inhibits the synthesis of serotonin, intraventricular infusion of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), which destroys the serotonergic cell bodies located in the raphe nuclei, and peripheral administration of the serotonergic neurotoxin *p*-chloroamphetamine (PCA), which destroys the serotonergic nerve terminals (2).

The serotonergic system has been proposed to be involved in the activation of the cerebral cortex (15,33,35,47,48). Depletion of brain serotonin either by systemic administration of *p*-CPA or intraventricular administration of 5,7-DHT abolishes atropine-resistent (serotonin dependent) low-voltage fast activity in the neocortex and rhythmic slow activity in the

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hippocampus (47,48). Based upon behavioral studies, Vanderwolf et al. proposed that impairments in performance of animals in tasks assessing learning or memory following serotonin depletion in the forebrain would more likely be due to a generalized deficit in behavior, that is, due to impaired control of motor activities by sensory stimuli, rather than learning or memory (47,48). However, many studies have attempted to define the role of the serotonergic system in learning and memory processes (2,3,4,11,18,21,22,24,28,29,34,36,41,43, 45,46,47,48). Nevertheless, the precise nature of its role is still unclear (2). The results of studies investigating the effects of partial depletion of brain serotonin on learning and memory have been inconsistent, depending upon the task used to assess learning or memory and the procedure used to lower brain serotonin concentrations (2). Indeed, partial depletion of brain serotonin has been found to either slightly impair, have no effect, or under certain conditions even facilitate learning of animals assessed using different maze or discrimination tasks (2,3,4,21,24,28,36,41,47,48). Further, memory retention assessed using, for example, different avoidance paradigms has also been found to be either impaired, improved, or without any effect after partial depletion of brain serotonin or pharmacological blockade of 5-HT receptors (2,11,18,22,29, 43,45,46,47,48). However, the serotonergic system also plays an important role in nociception (22,29,37,43,45) and conclusions from avoidance paradigm data are difficult to interpret. Further, the interpretation of the results of the avoidance paradigms may be confused by behaviorally effective variables such as arousal, motivation, and emotion (39), as well as motor abilities or perceptual processes (29).

Recently, continuous operant chamber tests analogous to those used for primates have been developed to test working (short-term, episodic) memory in rodents (14,30,40). These continuous operant chamber tasks allow dissociation between nonmnemonic and mnemonic changes in the performance of rats. Recent studies have shown that the same neural substrates, such as the prefrontal cortex and hippocampus, as well as some subcortical nuclei related to them, are involved in working memory of both primates (16) and rodents (1,14).

To study the role of the serotonergic system in higher cerebral functions, we are currently systemically investigating the effects of serotonin depletion in brain induced by systemic *p*-CPA administration on performance of rats in different behavioral tasks (20,21). In the present study, we were interested in studying the possible involvement of the serotonergic system in working memory. Therefore, the effects of *p*-CPA on the performance of adult rats in the delayed nonmatching to position task (DNMTP) (14,40) measuring spatial working memory were studied. Previously, Sakurai and Wenk found no effect with methysergide, a 5-HT_2 and 5-HT_1 receptor antagonist (8), on the performance of rats in a nonspatial working memory task (41). To further characterize the involvement of different 5-HT receptor subtypes in spatial working memory, the effects of methysergide, which has been used also in previous studies in our laboratory (34), on the performance of rats in the present DNMTP task were screened.

METHOD

Animals

Male Wistar rats (National Animal Center, Kuopio, Finland) were used in the present experiments. At the beginning of training, rats were 11 weeks old and at the beginning of the drug testing of the present experiment were 30 weeks old. Rats were housed singly in Makrolon cages in a temperature (20°C), humidity- (50–60%) and light period- (light on 0700–2100 h) controlled room. During training and testing, rats were food deprived 23 h before behavioral training and the experiment. After daily training, rats received about 10 g food pellets. Rats had free access to water except when in the operant chamber.

Apparatus

Testing was conducted in two operant chambers equipped with two retractable levers and a food dispenser (Campden Instruments, London, UK). The operant chambers were under the online control of microprocessors (Paul Fray Ltd., Cambridge, UK) programmed using SPIDER (Paul Fray Ltd.). The food dispenser deliver 45-mg pellets (Campden) (44).

Training Schedule

Rats were habituated to the chambers with the two retractable levers retracted and trained to collect food pellets and associate the click of the dispenser plus illumination of the panel light with pellet delivery. During this training (phase 1), a pellet was delivered every time a rat made a nose-poke into an illuminated pellet magazine. If the rat did not react within 20 s the illumination of magazine was turned off for 5 s. Rats were trained 10 min/day until they learned to obtain at least two pellets/min. In the next phase (phase 2), rats learned to associate the pressing of a lever with delivery of a food pellet. Both levers were inserted and each time a rat pressed a lever a food pellet was delivered into the magazine, which was illuminated. If the rat did not respond within 20 s, the levers were retracted for 5 s. In phase 3, rats learned to press a lever (either right or left) when it was inserted into the chamber to get a food pellet. The right or left lever was inserted randomly, and if the rat pressed the lever a food pellet was delivered and the magazine was illuminated. Then, the lever was retracted and after a 5-s period one of the levers was inserted once again. If the rat did not press the lever within 20 s, the lever was retracted and the houselight was turned off for 5 s.

In the next phase, rats were trained for nonmatching to position task (0-delay). A right or left lever (cue), selected randomly, was inserted into an operant chamber. When the rat pressed the lever, it was retracted and a magazine was illuminated but no food pellet was delivered. When the rat made a nose-poke into a magazine, the magazine light was turned off and both levers were inserted. In this choice phase, the pressing of the noncue lever was reinforced with delivery of a food pellet into the illuminated magazine. If the rat pressed the cue lever, the houselight was turned off for 5 s. After a 5-s period, a new cue lever was inserted. If the rat did not press the cue lever (omission 1) or one of the choice levers (omission 2) within 20 s, the houselight was turned off for 5 s and a new cue lever was inserted after a 5-s interval.

After rats (n = 18) had been trained for eight to nine times in the nonmatching to position task, delays (0, 1, 2, 4, 8, and 16 s) before inserting the choice levers were included in this nonmatching task (DNMTP). During each training session of the DNMTP task, all delays, which were introduced randomly, were used (about 10 trials/delay). The training (20 min/day) continued for 9 training days (2 training days/week) before tests were preformed. Then, 12 rats were trained in the DNMTP task using longer delays (0, 2, 4, 8, 16, and 30 s) for 9 training days. After this training, these rats were tested in the DNMTP task after acute treatments of saline, tacrine, an anticholinesterase, and zacopride, a 5-HT_3 receptor antagonist

(Jäkälä et al., submitted). The rest of the rats (n = 6) were tested using shorter delays (0–16 s) all the time during the experiments. After the tacrine/zacopride experiment, all rats (n = 18) were trained in a DNMTP task using shorter delays (0–16 s) six times during a 3-week wash-out period. Then, all rats were used for the present experiments. In the analysis of data, using multivariance analysis, the type of training (whether or not a rat was included in the tacrine/zacopride experiment) was taken into consideration, but it did not explain the treatment effects in the present experiment (data not shown in the results).

Drug Tests

Following the training period, the effects of methysergide (1.0, 5.0, and 15.0 mg/kg, IP; 4 ml/kg) on DNMTP performance were tested. Saline and the different doses of methysergide were tested in a counterbalanced order every second day. The injections were given 30 min before testing. Rats were allowed to adapt to the testing apparatus for a few minutes before testing. The percent correct total responses including all delays and the total number of trials completed (correct + incorrect) were taken for behavioral analysis. During this phase, one rat died.

One week after this part of the study, nine rats were treated with *p*-CPA (500 mg/kg/day × 3) and eight with arabic gum (AG) (500 mg/kg/day × 3) for control purposes. Six rats included in the tacrine/zacopride experiment were treated with *p*-CPA and six rats with AG. Injections were made IP (4.0 ml/kg) on each of 3 successive days. Three rats treated with *p*-CPA showed signs of illness (i.e., did not eat all their daily food pellets) and were excluded from the behavioral testing. Three days after the last injections of *p*-CPA or AG, rats were tested on concomitant days twice without any injections. The percent correct responses made by rats at each delay (0–16 s) were taken for behavioral analysis.

After this, the effects of saline and 2.0 mg/kg methysergide were tested in a counterbalanced order. The dose of 2.0 mg/kg methysergide was chosen because at the dose of 1.0 mg/kg methysergide alone did not have any clear-cut effects on the performance of rats whereas at the dose of 5.0 mg/kg the effects were already so severe that 4 of 17 rats made no responses during testing. Rats were treated 30 min before behavioral testing and allowed to adapt to the testing apparatus for a few minutes before testing. All variables [the percent correct responses, the total number of trials completed, omissions (during cue or choice), the latencies to correct or incorrect responses, and the latencies of sample press, nose-poke, and food collection] were used for behavioral analysis.

Neurochemical Analysis

For the monoamine and metabolite assays, tissue samples were weighed and homogenized (Potter, 10 times, 1,000 rpm) (Braun, Germany). Previously, we demonstrated that the changes in concentrations of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA), and dopamine (DA) induced by *p*-CPA (400 mg/kg/day × 3) treatment did not differ between frontal cortical, parietooccipito cortical, and hippocampal samples (20,21). Therefore, only frontal cortical and hippocampal monoamine and metabolite concentrations were determined. For determination of catecholamines, 65 μl tissue homogenate, 7.0 μl 2.5 μM DHBA in 0.2 M $HC10_4$, and 1 ml 0.5 M Tris-buffer, pH 8.6, were mixed and added to 20 mg $A1_20_3$. After that, tubes were mixed (15 min, at 4°C), centrifuged, and supernatant was aspirated off. The $A1_20_3$ was washed twice with 1 ml H_2O and amines were eluted with 70 μl 0.2 M $HC10_4$, of which 30 μl was injected into the chromatographic system. For determination of indoleamines and metabolites, 30 μl tissue homogenate and 5 μl 5 μM MOPET in 0.2 M $HC10_4$ were mixed and centrifuged 10 min at +4°C. Twenty microliters of the supernatant was injected into the chromatographic system. Chromatography was performed with an Hitachi L-6200L pump (Merck, Darmstadt, Germany) fitted with an Hitachi AS-4000 autosampler. Compounds were separated on C18 column (Beckman, Ultrasphere, 5-μm particle size, 4.6 mm × 25 cm Beckman Instruments, Fullerton, CA) using isocratic elution with a mobile phase consisting of 0.10 M sodium acetate, 0.10 M citric acid, 90 mg/l sodium octyl sulfate, and 20% MeOH at a flow rate of 0.8 ml/min for catecholamines and 1.5 ml/min for indoleamines and metabolites. An ESA detector 5100A (ESA, Inc.) with a 5011 dual analytic cell was used for electrochemical detection of eluting compounds. Detector 1 was set at +0.02 V and detector 2 at +0.35 V. Peaks were analyzed with HPLC-Manager software (Merck).

Statistical Analysis

Multivariate analysis of variance (MANOVA) or analysis of variance (ANOVA) were used to compare the group, treatment, and delay effects on the performance of rats. Before analysis, the data for percent correct responses were transformed using arcsine transformation, the data for omissions transformed using square root transformation, and the data for latencies transformed using logarithmic transformation. Neurochemical data were analyzed using ANOVA test.

RESULTS

Behavior

Effects of methysergide. Methysergide at the doses of 1.0, 5.0, and 15.0 mg/kg significantly decreased the percent correct total responses (all delays included) made by rats (Table 1). This decline in the percent correct responses was delay independent at 1.0 mg/kg methysergide (Table 1).

The total number of trials completed (correct + incorrect) was significantly decreased by all doses of methysergide (Table 1).

Effects of p-*CPA.* There was no difference in the percent correct responses between *p*-CPA-treated and control rats (Fig. 1). Increasing the duration of the delay significantly decreased the percent correct responses in both *p*-CPA-treated and control groups to the same extent (Fig. 1).

Methysergide at 2.0 mg/kg decreased the percent correct total responses in both *p*-CPA-treated and control groups to the same extent (Fig. 2). This decline in the percent correct responses induced by methysergide at 2.0 mg/kg was delay independent (Fig. 2).

In the analysis of the total number of trials completed, it was observed that *p*-CPA-treated and control rats did not differ between each other but that methysergide at 2.0 mg/kg significantly decreased the total number of trials in both *p*-CPA and control groups to the same extent (Table 2).

The numbers of omission 1 (during cue) were not affected by *p*-CPA treatment but were decreased by methysergide at 2.0 mg/kg (Table 2). The numbers of omission 2 (during choice) were increased by *p*-CPA treatment but not affected by methysergide 2.0 mg/kg (Table 2).

In the analysis of the latencies to correct or incorrect re-

TABLE 1

PERCENT CORRECT TOTAL RESPONSES INCLUDING ALL DELAYS (0–16 s) AND THE TOTAL NUMBER OF TRIALS COMPLETED OF SALINE AND METHYSERGIDE- (MET 1.0, 5.0, AND 15.0 mg/kg) TREATED RATS IN DELAYED NONMATCHING TO POSITION TASK

	% Correct Responses	Trials
Saline	76.1 ±1.7 (n = 17)	67.7 ± 2.2 (n = 17)
MET 1.0	70.2 ± 1.6 (n = 17)	55.7 ± 2.6 (n = 17)
MET 5.0	60.0 ± 4.1 (n = 13)	20.5 ± 4.6 (n = 17)
MET 15.0	53.3 ±2.2 (n = 4)	5.1 ± 2.2 (n = 17)

Values are expressed as group means ± SEM. The means ± SEM for % correct responses have been calculated for rats that made at least four trials during a 20-min testing session. The number in parentheses indicates the number of rats/group. Four rats treated with Met 5.0 and 13 rats treated with Met 15.0 made no responses during testing. Statistical analysis (MANOVA followed by Wilcoxon signed rank test): Analysis of the % correct responses revealed a significant treatment effect, $F(3, 45) = 37.4$, $p < 0.001$. Methysergide-treated rats performed more poorly than saline-treated rats: Met 1.0 vs. saline, $Z(14, 3) = -2.53$, $p < 0.02$; Met 5.0 vs. saline, $Z(16, 1) = -2.96$, $p < 0.005$; Met 15.0 vs. saline, $Z(17, 0) = -3.62$, $p < 0.001$. At 1.0 mg/kg methysergide, delay effect, $F(5, 96) = 32.3$, $p < 0.001$, and methysergide treatment effect, $F(1, 96) = 7.28$, $p < 0.01$, were significant, but there was no interaction between delay and treatment, $F(5, 96) = 0.75$, $p > 0.1$. Analysis of the number of trials revealed a significant treatment effect, $F(3, 45) = 81.7$, $p < 0.001$. Methysergide-treated rats made fewer trials than saline-treated rats: Met 1.0 vs. saline, $Z(13, 3) = -2.95$, $p < 0.005$; Met 5.0 vs. saline, $Z(16, 1) = -3.52$, $p < 0.001$; Met 15.0 vs. saline, $Z(17, 0) = -3.62$, $p < 0.001$.

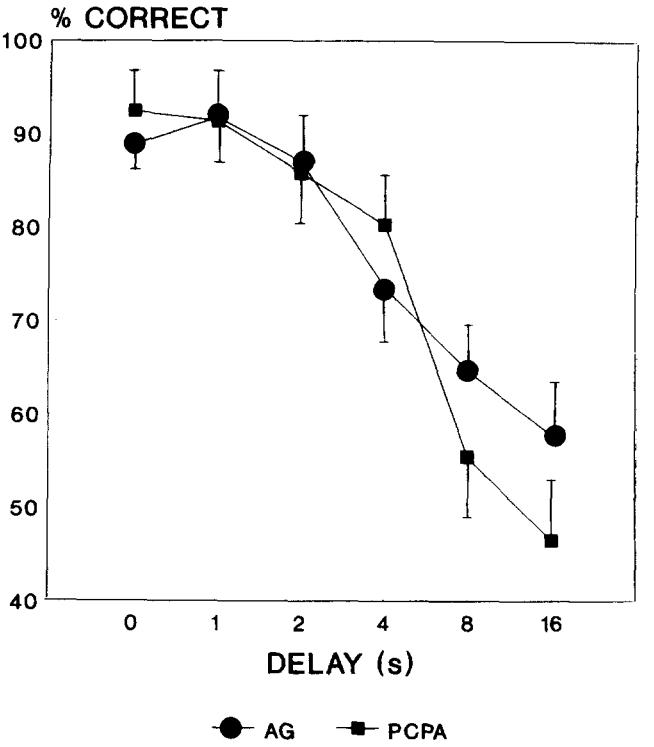

FIG. 1. Percent correct responses at each delay (0–16 s) of p-chlorophenylalanine- (p-CPA) (500 mg/kg/day × 3, IP) (n = 6) treated rats. Arabic gum (AG) (500 mg/kg/day × 3, IP) (n = 8) treatment served as control for p-CPA treatment. The percent correct responses (% CORRECT) have been calculated as correct responses/correct responses + incorrect responses × 100 and are expressed as a mean from two testing sessions 3 and 4 days after the last injections of p-CPA or AG. The vertical bars indicate ±SEM. Statistical analysis was made using analysis of variance (group and delay as variables). The effect of delay was significant, $F(5, 7) = 22.6$, $p < 0.001$. The group effect did not reach significance, $F(1, 7) = 0.003$, $p > 0.1$, and there was no interaction between the delay and groups, $F(5, 11) = 0.89$, $p > 0.1$

sponses, no significant p-CPA or methysergide 2.0 mg/kg treatment effect was revealed (Table 2).

The latency of sample press was significantly increased by p-CPA (Table 2). Methysergide at 2.0 mg/kg significantly increased the latency of sample press in both p-CPA-treated and control groups to the same extent (Table 2).

The latency of nose-poke was not affected by p-CPA treatment but was significantly and equally increased by methysergide 2.0 mg/kg in both p-CPA-treated and control rats (Table 2).

The latency to collect food pellets was significantly increased by p-CPA (Table 2). Methysergide 2.0 mg/kg slightly increased the food collection latency in both p-CPA treated and control rats to the same extent (Table 2).

Neurochemical Analysis

p-CPA (500 mg/kg/day × 3, IP) treatment significantly depleted the frontal cortical and hippocampal concentrations of both serotonin and its major metabolite 5-HIAA when measured 7 days after the last injection of p-CPA. Biochemical analysis revealed that the frontal cortical and hippocampal concentrations of NA and DA were also slightly decreased by p-CPA treatment (Table 3).

DISCUSSION

The present study investigated the effects of serotonergic dysfunction on working memory. Therefore, the effects of serotonin synthesis inhibition induced by p-CPA and pharmacological blockade of 5-HT receptors induced by methysergide on the performance of rats in the DNMTP task assessing spatial working memory were studied.

p-CPA treatment induced an almost total depletion of frontal cortical and hippocampal serotonin and its major metabolite 5-HIAA and slightly decreased behavioral activity of rats, as indicated by increased latencies and the tendency towards decreased number of trials completed. Further, when analyzed before testing with 2.0 mg/kg methysergide p-CPA significantly decreased the number of trials completed (data not shown). This finding of decreased behavioral activity is in line with previous studies (20,47,48). However, also frontal cortical DA and hippocampal NA concentrations were decreased by p-CPA treatment. Dopaminergic dysfunction might affect response initiation and vigor (13), and it cannot be excluded that the decrease in DA concentration in the frontal cortex or some other brain area(s), such as, for example, the striatum, where DA concentrations were not analyzed in the present study, might at least partly account for the decreased behavioral activity induced by p-CPA treatment. p-CPA also significantly increased the latency to collect earned

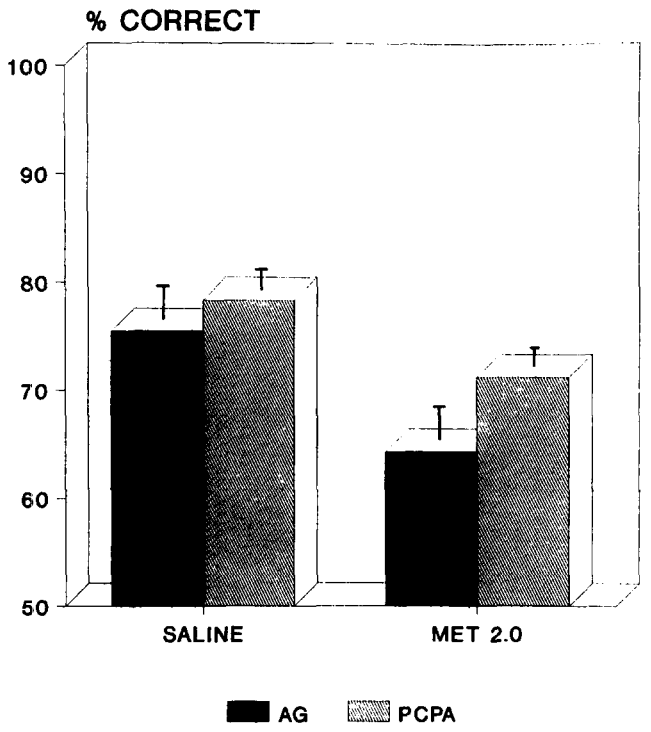

FIG. 2. Percent correct total responses including all delays (0–16 s) of *p*-chlorophenylalanine-(*p*-CPA) (500 mg/kg/day × 3, IP) (n = 6) treated rats following saline or methysergide (2.0 mg/kg) administration. Arabic gum (AG) (500 mg/kg/day × 3, IP) (n = 8) treatment served as control for *p*-CPA treatment. Methysergide and saline were administered (4.0 ml/kg, IP) 30 min before testing. For the calculation of the percent correct responses, see Fig. 1. Vertical bars indicate group means ± SEM. Statistical analysis were made using multivariate analysis of variance (group, treatment, and delay as variables). The group effect was nonsignificant, $F(1, 10) = 0.64$, $p > 0.1$. The treatment effect was slight, $F(1, 10) = 4.50$, $p = 0.06$, and there was no interaction between groups and treatments, $F(1, 10) = 0.02$, $p > 0.1$. The effect of delay was significant, $F(5, 60) = 13.8$, $p < 0.001$, but there was no interaction between delay and groups, $F(5, 60) = 0.95$, $p > 0.1$, or between delay and treatments, $F(5, 60) = 0.90$, $p > 0.1$.

food pellets after a correct response, suggesting that *p*-CPA might have affected the motivation of rats for food reward. Previously, it was suggested that ICV *p*-CPA treatment may induce hyperphagia with a concomitant increase in body weight (7), whereas systemic *p*-CPA treatment was suggested to produce a variety of changes in food consumption, some of which may depend upon intestinal disturbances (7). However, anorectic actions have most commonly been described for 5-HT agonistic drugs (7,42).

Interestingly, almost total depletion of brain serotonin by *p*-CPA as assessed in the frontal cortex and hippocampus had no effect on the percent correct responses, suggesting that brain serotonin may not necessarily be crucial for spatial working memory of rats as assessed using the present continuous DNMTP task. However, it could be speculated that some changes occurring in other neurotransmitter systems, such as increased turnover of NA (31) or acetylcholine (19,38), might compensate for some of the behavioral consequences following brain serotonin depletion. Previously, Altman et al. reported enhanced acquisition and performance of rats in a

TABLE 2

TOTAL NUMBER OF TRIALS, OMISSIONS AT THE TIME TO RESPOND TO CUE (OMISSION 1) AND CHOICE (OMISSION 2), LATENCY TO CORRECT AND INCORRECT RESPONSES, AND LATENCY OF SAMPLE PRESS, NOSE-POKE, AND FOOD COLLECTION OF *p*-CHLOROPHENYLALANINE-(500 mg/kg/day × 3, IP) TREATED AND CONTROL RATS FOLLOWING SALINE OR METHYSERGIDE TREATMENT

	Saline	MET 2.0
Trials		
AG (n = 8)	67.3 ± 3.0	31.6 ± 7.1
p-CPA (n = 6)	53.8 ± 4.5	31.3 ± 5.9
Omission 1		
AG	10.4 ± 3.9	4.4 ± 3.8
p-CPA	7.3 ± 1.7	7.8 ± 3.2
Omission 2		
AG	0.1 ± 0.2	0
p-CPA	0.2 ± 0.1	0.3 ± 0.4
Correct		
AG	0.48 ± 0.11	0.75 ± 0.11
p-CPA	0.72 ± 0.19	0.76 ± 0.24
Incorrect		
AG	0.71 ± 0.14	0.76 ± 0.17
p-CPA	1.19 ± 0.14	1.62 ± 0.54
Sample Press		
AG	2.49 ± 0.13	4.15 ± 0.45
p-CPA	4.77 ± 0.83	7.09 ± 0.85
Nose-poke		
AG	1.01 ± 0.11	4.58 ± 1.30
p-CPA	1.75 ± 0.51	4.01 ± 1.69
Food collection		
AG	0.53 ± 0.03	0.64 ± 0.10
p-CPA	1.02 ± 0.22	1.50 ± 0.55

Results are expressed as means ± SEM. The number in parentheses indicates the number of rats/group. The latencies are expressed as seconds. Statistical analysis using MANOVA [group (*p*-CPA/AG) and treatment (saline/methysergide 2.0 mg/kg) as variables]: *Trials:* Group effect was nonsignificant, $F(1, 10) = 0.58$, $p > 0.1$. Treatment effect was significant, $F(1, 10) = 25.9$, $p < 0.001$, and there was no interaction between groups and treatments, $F(1, 10) = 0.84$, $p < 0.1$. *Omission 1:* Group effect was nonsignificant, $F(1, 10) = 0.34$, $p < 0.1$. Treatment effect was significant, $F(1, 10) = 8.56$, $p < 0.05$, and there was no interaction between groups and treatments, $F(1, 10) = 4.16$, $p > 0.05$. *Omission 2:* Group effect was significant, $F(1, 10) = 8.24$, $p < 0.05$. Treatment effect was nonsignificant, $F(1, 10) = 0.02$, $p > 0.1$, and there was no interaction between groups and treatments, $F(1, 10) = 1.88$, $p > 0.1$. *Latency to a correct response (CORRECT):* Group effect was nonsignificant, $F(1, 10) = 0.00$, $p > 0.1$. Treatment effect was nonsignificant, $F(1, 10) = 1.42$, $p > 0.1$, and there was no interaction between groups and treatments, $F(1, 10) = 1.43$, $p > 0.1$. *Latency to an incorrect response (INCORRECT):* Group effect was nonsignificant, $F(1, 10) = 2.27$, $p > 0.1$. Treatment effect was also nonsignificant, $F(1, 10) = 0.27$, $p > 0.1$, and there was no interaction between groups and treatments, $F(1, 10) = 1.60$, $p > 0.1$. *Sample press:* Group effect was significant, $F(1, 19) = 13.2$, $p < 0.02$. Treatment effect was significant, $F(1, 10) = 16.0$, $p < 0.01$, and there was no interaction between groups and treatments, $F(1, 10) = 0.04$, $p > 0.1$. *Nose-poke:* Group effect was nonsignificant, $F(1, 10) = 0.05$, $p > 0.1$. Treatment effect was significant, $F(1, 10) = 7.13$, $p < 0.05$, and there was no interaction between groups and treatments, $F(1, 10) = 0.65$, $p > 0.1$. *Food collection:* Group effect was significant, $F(1, 10) = 6.48$, $p < 0.05$. Treatment effect was slight, $F(1, 10) = 5.43$, $p = 0.06$, and there was no interaction between groups and treatments, $F(1, 10) = 0.36$, $p > 0.1$.

TABLE 3

EFFECTS OF *p*-CHLOROPHENYLALANINE (500 mg/kg/day × 3, IP) TREATMENT ON THE CONCENTRATIONS OF FRONTAL CORTICAL AND HIPPOCAMPAL MONOAMINES AND METABOLITES

	NA	DA	5-HT	5-HIAA
AG				
fc	285.0 ± 34.2	74.5 ± 10.6	575.4 ± 51.5	302.9 ± 16.4
hipp	557.2 ± 23.7	n.d.	395.9 ± 45.9	503.1 ± 43.8
p-CPA				
fc	199.5 ± 18.6	42.7 ± 6.2	15.3 ± 1.3	2.4 ± 1.2
hipp	313.5 ± 12.0	n.d.	1.7 ± 1.9	5.6 ± 2.1

Values (ng/g brain tissue) are expressed as group means ± SEM. AG, arabic gum (500 mg/kg/day × 3, IP) (n = 8); DA, dopamine; fc, frontal cortex; hipp, hippocampus; NA, noradrenaline; *p*-CPA, *p*-chlorophenylalanine (500 mg/kg/day × 3, IP) (n = 6); 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine. Statistical analysis by ANOVA, group effects: frontal cortical noradrenaline, $F(1, 13) = 4.58, p > 0.05$; frontal cortical dopamine, $F(1, 13) = 6.46, p < 0.05$; frontal cortical 5-HIAA, $F(1, 13) = 280, p < 0.001$; frontal cortical 5-HT, $F(1, 13) = 99.3, p < 0.001$; hippocampal noradrenaline, $F(1, 13) = 68.1, p < 0.001$; hippocampal dopamine, not determined (n.d.); hippocampal 5-HIAA, $F(1, 13) = 108, p < 0.001$; hippocampal 5-HT, $F(1, 13) = 25.1, p < 0.001$.

complex positively reinforced spatial discrimination task following partial reductions in brain serotonin after either PCA-induced destruction of 5-HT terminals (4) or 5,7-DHT-induced serotonergic deafferentation of the hippocampus (3). On the other hand, the performance of PCA-treated rats trained in another complex positively reinforced spatial discrimination task, that is, eight-arm radial arm maze, was not different from controls (4). Further, in a study by Sakurai and Wenk partial serotonin depletion induced by PCA did not affect the performance of rats in a nonspatial working memory task, that is, continuous nonmatching to sample task (41). However, it is of interest that in many studies serotonergic dysfunction alone has failed to affect or has had only a limited effect on performance of a learning or memory task but has interacted with other neurotransmitter systems, such as the cholinergic system (18,24,28,34,36,41,47,48). Most commonly, aggravation of an impairment in the performance of a task proposed to assess learning or memory induced by cholinergic dysfunction has been reported after partial brain serotonin depletion or serotonergic lesions (18,24,28,36,47, 48), although this serotonergic dysfunction alone has failed to affect or has had only a slight effect on performance of such a task. However, in the study by Sakurai and Wenk partial brain serotonin depletion by PCA significantly diminished the performance impairment produced by scopolamine in a nonspatial working memory task (41).

Methysergide (1.0–15.0 mg/kg) induced a disruption in the percent correct responses. However, this disruption was delay independent. Therefore, it is not reasonable to conclude that methysergide affected working memory of rats. Further, methysergide significantly decreased the behavioral activity of rats. In the study by Markowska and Wenk, methysergide (5–15 mg/kg) also impaired performance of rats in a nonspatial memory task (24). On the other hand, in another study methysergide at 15.0 or 30.0 mg/kg did not impair performance of rats in a nonspatial working memory task, that is, continuous nonmatching to sample task in an operant chamber (41). In these studies, it was not reported whether methysergide affected behavioral activity as was seen in the present task. Previously, in our laboratory methysergide (1.0, 5.0, or 15.0 mg/kg) did not affect the speed of swimming or learning of rats in a water maze spatial navigation task (34) and, further, in the present study it was observed that after all doses of methysergide rats were walking around and exploring the test chamber. Therefore, it is plausible that the decreased behavioral activity seen after methysergide treatment was not due to akinesia. It is also unlikely that methysergide would have affected motivation for food reward because: a) The latency to collect food reward after a correct response was only slightly affected by methysergide treatment; b) the number of omissions were not increased by methysergide; and c) anorectic actions have most commonly been described for 5-HT agonistic drugs (7,42). Therefore, it is unlikely that methysergide would have affected motor ability or motivational factors of rats in the present task. Interestingly, cortical desynchronization induced by noxious stimuli has been demonstrated to be abolished by systemic or local application of serotonin receptor antagonists to the cortex (27). Further, methysergide potentiates the depressant effects of serotonin either in the somatosensory (10) or prefrontal cortex (23) and antagonizes the excitatory effects of serotonin in the somatosensory cortex (10) in rats. Therefore, it is possible that the disruption of performance of rats in the present task following methysergide treatment may be attributable to a decreased level of cortical arousal.

Interestingly, *p*-CPA and methysergide did not produce similar effects on the performance of rats in the task. Based upon the above discussion, it may be speculated that the effects of methysergide may be due to antagonism of excitation and potentiation of inhibition produced by serotonin whereas after almost total depletion of brain serotonin by *p*-CPA neither excitatory nor inhibitory responses to serotonin could be observed.

One curious finding of the present study was that the effects of methysergide were not diminished by *p*-CPA treatment, which induced almost total depletion of brain serotonin. A possible explanation for this unexpected finding is that the depletion in brain serotonin, although almost total in the frontal cortex and hippocampus, induced by *p*-CPA was not severe enough in some other brain area(s) or the periphery to prevent the effects of methysergide. Another possible explana-

tion is that the effects of methysergide on the present task may not be the result of a central antiserotonergic action. It has been suggested that methysergide may not be a selective antagonist of the central serotonergic system (17). Indeed, the behavioral activity-decreasing effects of methysergide could indicate an antidopaminergic action because dopaminergic dysfunction may disrupt response initiation and vigor (13). However, as mentioned above, in the present study methysergide-treated rats were not akinetic and previously in our laboratory methysergide did not affect the performance of rats in a water maze test (34). Further, DA depletion in the prefrontal cortex in monkeys has been shown to induce delay-dependent short-term memory deficits in a delayed-alternation task (12), whereas in the present study methysergide induced a delay-independent disruption of percent correct responses. With regard to the serotonergic system, methysergide is usually considered as an antagonist at 5-HT_2 receptors (8). However, methysergide may also possess affinity for 5-HT_1 receptors (8). Therefore, in future studies more selective antagonists at central 5-HT_2 receptors, such as ketanserin (8), should be used to test whether the effects of methysergide on the performance of the present task were due to antagonism at 5-HT_2 binding sites.

The results of the present study do not support any important role for the serotonergic system in working memory and therefore do not suggest that serotonergic degeneration alone would contribute to AD-related working (episodic) memory deficit (26). Interestingly, administration of a selective serotonin reuptake blocker, alaproclate, has been reported to improve the capacity to cope with life in some AD patients (6). However, these improvements may be attributable to enhanced emotional function rather than alleviation of memory deficits per se (18). Investigations of interactions of the serotonergic system with other neurotransmitter systems, such as with cholinergic neurons, on the performance of rats in the present working memory, continuous DNMTP task may help clarify the background of the AD-related short-term memory deficit.

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REFERENCES

1. Aggleton, J. P.; Keith, A. B.; Sahgal, A. Both fornix and anterior thalamic, but not mamillary, lesions disrupt delayed non-matching-to-position memory in rats. Behav. Brain Res. 44:151–161; 1991.
2. Altman, H. J.; Normile, H. J. What is the nature of the role of the serotonergic nervous system in learning and memory: Prospects for development of an effective treatment strategy for senile dementia. Neurobiol. Aging 9:627–638; 1988.
3. Altman, H. J.; Normile, H. J.; Galloway, M. P.; Ramirez, A.; Azmitia, E. Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. Brain Res. 518:61–66; 1990.
4. Altman, H. J.; Ögren, S. O.; Berman, R. F.; Normile, H. J. The effects of *p*-chloroamphetamine, a depletor of brain serotonin, on the performance of rats in two types of positively reinforced complex spatial discrimination tasks. Behav. Neural. Biol. 52: 131–144; 1989.
5. Bartus, R. T. The need for common perspectives in the development and use of animal models for age-related cognitive and neurodegenerative disorders. Neurobiol. Aging 9:445–451; 1988.
6. Bergman, I.; Brane, G.; Gottfries, C. G.; Jostell, K.-G.; Karlsson, I.; Svennerholm, L. Alaproclate: A pharmacokinetic and biochemical study in patients with dementia of the Alzheimer type. Psychopharmacology (Berl.) 80:279–283; 1983.
7. Blundell, J. E. Serotonin and appetite. Neuropharmacology 23: 1537–1551; 1984.
8. Bonate, P. L. Serotonin receptor subtypes: Functional, physiological, and clinical correlates. Clin. Neuropharmacol. 14:1–16; 1991.
9. Bowen, D. M.; Allen, S. J.; Benton, J. S.; Goodhardt, M. J.; Haan, E. A.; Palmer, A. M.; Sims, N. R.; Smith, C. C. T.; Spillane, J. A.; Esira, G. K.; Neary, D.; Snowden, J. S.; Wilcock, G. K.; Davison, A. N. Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. J. Neurochem. 41:266–272; 1983.
10. Bradshaw, C. M.; Stoker, M. J.; Szabadi, E. Comparison of neuronal responses to 5-hydroxytryptamine, noradrenaline, and phenylephrine in the cerebral cortex: Effects of haloperidol and methysergide. Neuropharmacology 22:677–683; 1983.
11. Brody, J. F. Behavioural effects of serotonin depletion and of *p*-chlorophenylalanine (a serotonin depleter) in rats. Psychopharmacologia 17:14–33; 1970.
12. Brozoski, T.; Brown, R. M.; Rosvold, H. E.; Goldman, P. S. Cognitive deficit caused by depletion of dopamine in prefrontal cortex of rhesus monkey. Science 205:929–931; 1979.
13. Cole, B. J.; Robbins, T. W. The effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: Implications for theories of selective attention and arousal. Behav. Brain Res. 33:165–179; 1989.
14. Dunnett, S. B. Role of prefrontal cortex and striatal output systems in short-term memory deficits associated with aging, basal forebrain lesions, and cholinergic-rich grafts. Can. J. Psychol. 44:210–232; 1990.
15. Foote, S. L.; Morrison, J. H. Extrathalamic modulation of cortical function. Annu. Rev. Neurosci. 10:67–95; 1987.
16. Goldamn-Rakic, P. S. Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In: Blum, F., ed. Handbook of physiology. Bethesda, MD: Americal Physiology Society; 1987:373–417.
17. Haigler, H. J.; Aghajanian, G. K. Peripheral serotonin antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotonergic input. J. Neural Trans. 35:257–273; 1974.
18. Haroutunian, V.; Santucci, A. C.; Davis, K. L. Implications of multiple transmitter system lesions for cholinomimetic therapy in Alzheimer's disease. In: Aquilonius, S.-M.; Gillberg, P.-G., eds. Progress in brain research. vol. 84. Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division); 1990:333–346.
19. Jackson, D.; Stachowiak, M. K.; Bruno, J. P.; Zigmond, M. J. Inhibition of striatal acetylcholine release by endogenous serotonin. Brain Res. 457:259–266; 1988.
20. Jäkälä, P.; Sirviö, J.; Jolkkonen, J.; Riekkinen, P., Jr.; Acsady, L.; Riekkinen, P. The effects of *p*-chlorophenylalanine induced serotonin synthesis inhibition and muscarinic blockade on the performance of rats in a 5-choice serial reaction time task. Behav. Brain Res. (in press).
21. Jäkälä, P.; Sirviö, J.; Riekkinen, P., Jr.; Valjakka, A.; Riekkinen, P. The effects of alaproclate and *p*-chlorophenylalanine on cued navigation performance in rats. J. Neural Trans. 4:43–52; 1992.
22. Koe, B. K.; Weissman, A. *p*-Chlorophenylalanine: A specific depletor of brain serotonin. J. Pharmacol. Exp. Ther. 154:499–516; 1966.

23. Lakoski, J. M.; Aghajanian, G. K. Effects of ketanserin on neuronal responses to serotonin in the prefrontal cortex, lateral geniculate and dorsal raphe nucleus. Neuropharmacology 24:265–273; 1985.
24. Markowska, A. L.; Wenk, G. L. Serotonin influences the behavioral recovery of rats following nucleus basalis lesions. Pharmacol. Biochem. Behav. 38:731–737; 1991.
25. Mesulam, M.-M. Large-scale neurocognitive networks and distributed processing for attention, language and memory. Ann. Neurol. 28:597–613; 1990.
26. Morris, R. G.; Kopelman, M. D. The memory deficits in Alzheimer-type dementia: A review. Q. J. Exp. Psychol. 38A:575–602; 1986.
27. Neuman, R. S.; Zebrowski, G. A. Activation of 5-HT2 receptors alters unit activity of cortical neurones. Soc. Neurosci. Abstr. 15: 554; 1989.
28. Nilsson, O. G.; Brundin, P.; Björklund, A. Amelioration of spatial memory impairment by intrahippocampal grafts of mixed septal and raphe tissue in rats with combined cholinergic and serotonergic denervation of the forebrain. Brain Res. 515:193–206; 1990.
29. Ögren, S.-O.; Johansson, C. Separation of the associative and non-associative effects of brain serotonin released by *p*-chloroamphetamine: Dissociable serotonergic involvement in avoidance learning, pain and motor function. Psychopharmacology (Berl.) 86:12–26; 1985.
30. Pontocorvo, M. J.; Clissold, D. B.; Conti, L. H. Age-related cognitive impairments as assessed with an automated repeated measures memory task: Implications for the possible role of acetylcholine and norepinephrine in memory dysfunction. Neurobiol. Aging 9:617–625; 1989.
31. Reader, T. A.; Briere, R.; Grondin, L.; Ferron, A. Effects of *p*-chlorophenylalanine on cortical monoamines and on the activity of noradrenergic neurons. Neurochem. Res. 11:1025–1035; 1986.
32. Reinikainen, K. J.; Soininen, H.; Riekkinen, P. J. Neurotransmitter changes in Alzheimer's disease: Implications to diagnostics and therapy. J. Neurosci. Res. 27:576–586; 1990.
33. Richter-Levin, G.; Segal, M. The effects of serotonin depletion and raphe grafts on hippocampal electrophysiology and physiology. J. Neurosci. 11:1585–1596; 1991.
34. Riekkinen, M.; Riekkinen, P., Jr.; Riekkinen, P. Effects of combined methysergide and mecamylamine/scopolamine treatment on spatial navigation. Brain Res. 585:322–326; 1992.
35. Riekkinen, P., Jr.; Sirviö, J.; Miettinen, R.; Riekkinen, P. Interaction between raphe dorsalis and nucleus basalis magnocellularis in the regulation of high-voltage spindle activity in rat neocortex. Brain Res. 526:31–36; 1990.
36. Riekkinen, P., Jr.; Sirviö, J.; Valjakka, A.; Miettinen, R.; Riekkinen, P. Pharmacological consequences of cholinergic plus serotonergic manipulations. Brain Res. 552:23–26; 1991.
37. Roberts, M. H. T. 5-Hydroxytryptamine and antinociception. Neuropharmacology 12:1529–1536; 1984.
38. Robinson, S. E. Effect of specific serotonergic lesions on cholinergic neurons in the hippocampus, cortex and striatum. Life Sci. 32:345–353; 1983.
39. Sahgal, A. Does vasopressin affect cerebral function: In: Baylis, P. H.; Padfield, P. L., eds. The posterior pituitary—hormone secretion in health and disease. New York: Marcel Dekker; 1985: 325–359.
40. Sahgal, A.; Keith, A. B.; Lloyd, S.; Kerwin, J. M.; Perry, E. K.; Edwardson, J. A. Memory following cholinergic (NBM) and noradrenergic (DNAB) lesions made by singly or in combination: Potentiation of disruption by scopolamine. Pharmacol. Biochem. Behav. 37:597–606; 1990.
41. Sakurai, Y.; Wenk, G. L. The interaction of acetylcholinergic and serotonergic neural systems on performance in a continuous non-matching to sample task. Brain Res. 519:118–121; 1990.
42. Samanin, R.; Garattini, S. The pharmacology of serotonergic drugs affecting appetite. In: Wurtman, R. J.; Wurtmann, J. J., eds. Nutrition and the brain. vol. 8. New York: Raven Press; 1990:163–192.
43. Schlesinger, K.; Schreiber, A.; Pryor, G. T. Effects of *p*-chlorophenylalanine on conditioned avoidance learning. Psychonom. Sci. 11:225–226; 1968.
44. Sirviö, J.; Lukkarinen, K.; Riekkinen, P., Jr.; Koivisto, E.; Virtanen, R.; Pennanen, A.; Valjakka, A.; Riekkinen, P. J. The effects of atipamezole, an alpha-2 antagonist, on the performance of young and aged rats in the delayed nonmatching to position task. Pharmacol. Biochem. Behav. 39:1015–1019; 1991.
45. Tenen, S. S. The effects of *p*-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behaviors in the rat. Psychopharmacologia 10:204–219; 1967.
46. Valzelli, L.; Pawlowski, L. Effects of *p*-chlorophenylalanine on avoidance learning of two differentially housed mouse strains. Neuropsychobiology 5:121–128; 1979.
47. Vanderwolf, C. H. Cerebral activity and behavior: Control by central cholinergic and serotonergic systems. Int. Rev. Neurobiol. 30:225–240; 1988.
48. Vanderwolf, C. H. A general role for serotonin in the control of behavior: Studies with intracerebral, 5,7-dihydroxytryptamine. Brain Res. 504:192–198; 1989.
49. Winblad, B.; Hardy, J.; Bäckman, L.; Nilsson, L.-G. Memory function and brain biochemistry in normal aging and senile dementia. Ann. NY Acad. Sci. 444:255–268; 1985.