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Excessive serotonin release, not depletion, leads to memory impairments in rats

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Abstract

Eight experiments compared and contrasted the effects of serotonin release and depletion on performance by rats in two tests of memory. Most experiments (Experiments 1–5) examined the effects of the serotonergic releasing/depleting agent *p*-chloroamphetamine on passive avoidance performance. Additional experiments explored *p*-chloroamphetamine's effects on retention performance by animals trained in an 8-arm radial maze (Experiment 6), and the effects of dorsal raphe nucleus lesions on passive avoidance in animals treated with (Experiment 8) or not treated with (Experiment 7) *p*-chloroamphetamine. In general, acute increases in serotonin release produced consistent and extensive retention performance deficits in both passive avoidance and radial arm maze. Results from an ancillary control experiment indicated that the *p*-chloroamphetamine-induced passive avoidance impairment was not related to drug-induced alterations in pain sensitivity. Other experiments ruled out the possibility that *p*-chloroamphetamine was disrupting passive avoidance retention performance by affecting post-trial consolidation processes, producing state-dependent retention, having direct effects at postsynaptic receptors, or indirectly by affecting non-serotonergic neurotransmitter systems. Depletion of serotonin resulting from either the long-term residual effects of *p*-chloroamphetamine or lesions of the dorsal raphe nucleus failed to alter passive avoidance retention scores although it produced extensive depletion (45–85%) of serotonin and 5-hydroxyindoleacetic acid in the cortex and hippocampus. These data contribute to the growing body of literature indicating an important role of serotonin in cognitive processes by demonstrating that excessive release, but not depletion, of serotonin produces profound retention performance impairment.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); *p*-Chloroamphetamine; Memory; Alzheimer's disease; (Rat)

1. Introduction

There has been increased attention focusing on the role of the serotonergic system in learning and memory over the past decade. There have been a great many reports regarding the existence of serotonergic perturbations associated with age-related cognitive disorders such as Alzheimer's disease. Postmortem studies of Alzheimer's disease brains have indicated decreases in the number of serotonergic cells (Curcio and Kemper, 1984; German et al., 1987; Ichimiya et al., 1986) and receptors (Cross et al., 1984; Cross et al., 1986), in addition to reductions in the levels of the neurotransmitter itself and its major metabolite, 5-hydroxyindoleacetic acid (Arai et al., 1984; Gottfries et al., 1989; Yates et al., 1986). Whether such alterations in serotonergic neurotransmission are causally linked to the decline in cognitive abilities, especially learning and memory, remains to be determined. However, the fact that clinical trials with cholinomimetics have shown only partial memory enhancement in Alzheimer's disease (see Mohs and Davis, 1987 for review) is consistent with the hypothesis that impairment of non-cholinergic systems, including the serotonergic system, plays an important role in the manifestation of cognitive symptoms.

Although there is growing evidence from animal experiments indicating a role for serotonin in modulat-

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ing learning and memory processes, this modulation is not well characterized. Both increases and decreases in performance have been reported following similar experimental manipulations of the serotonergic system. For example, injections of a variety of drugs which increase synaptic serotonin availability, such as *p*-chloroamphetamine (Ögren, 1986a, b; Ögren et al., 1985; Santucci et al., 1990), alaproclate (Altman et al., 1984, 1987; Riekkinen et al., 1991a), fenfluramine (Lalonde and Vikis-Freibergs, 1985), and fluoxetine (Altman et al. 1984; Flood and Cherkin, 1987; Lalonde and Vikis-Freibergs, 1985), have produced enhancement (Altman et al., 1984, 1987; Flood and Cherkin, 1987) and impairment (Lalonde and Vikis-Freibergs, 1985; Ögren, 1986a; Ögren et al., 1985; Riekkinen et al., 1991a; Santucci et al., 1990) in tests of learning and memory. Similarly, bidirectional changes have been observed when a variety of 5-HT antagonists were studied with both increases (Altman and Normile, 1986, 1987) and decreases (Altman and Normile, 1987; Jäkälä et al., 1993; Winter and Petti, 1987) in performance reported. Finally, some investigators have noted impaired performance following experimentally induced depletion of serotonin (Ögren et al., 1985; Richter-Levin and Segal, 1991), while others have found enhanced performance (Altman and Normile, 1988; Normile et al., 1990; Richter-Levin and Segal, 1991) or no change in behavior (Jäkälä et al., 1993; Richter-Levin and Segal, 1989; Markowska and Wenk, 1991; Riekkinen et al., 1990, 1991b, 1992, 1993; Riekkinen and Riekkinen, 1995; Santucci et al., 1995).

The role of serotonin in learning and memory is still unclear as results appear to depend on the cognitive process under investigation and/or the motivational characteristics of the task. For example, Altman and his colleagues often use a Stone maze to assess long-term retrieval processes with animals being trained for one daily trial over the course of several days (Altman and Normile, 1988; Normile et al., 1990). Using this procedure this group has demonstrated enhanced acquisition (as assessed by a lessened number of errors) following serotonergic depletion induced by the long-term neurotoxic effects of *p*-chloroamphetamine. In contrast, other researchers have found that *p*-chloroamphetamine-induced serotonergic depletion is ineffective to alter either the spatial working memory in rats trained in a Morris water maze (Santucci et al., 1995) or the choice accuracy of rats trained over 4 weeks with a multi-trial non-spatial visual discrimination memory task (Markowska and Wenk, 1991). There is similar confusion when the effects of serotonin release are assessed. Injections of *p*-chloroamphetamine in rats shortly prior to training, for example, have been reported to impair acquisition of active avoidance without affecting 24-h retention performance (Ögren, 1986b). In contrast, pre-test administration of *p*-chloroamphetamine impaired retrieval of an active avoidance task, an effect not seen when passive avoidance was the assessment instrument (Ögren, 1986b). Clearly, it is important to consider both the nature of the cognitive process and the characteristics of the task when assessing the role of serotonin in learning and memory.

Obviously, variations in methodologies, including differences in the types of subjects used, tests employed, pharmacological agents administered, and timing of treatments, all contribute to the inconsistencies and often confusion, in the results. We therefore aimed to clarify the role of serotonin by conducting a comprehensive investigation using similar methodologies throughout. The aim of the eight experiments reported here was to compare the effects of serotonergic release and depletion on learning and memory in rats. The effects of *p*-chloroamphetamine, a neurotoxin which produces short-term release of serotonin (Sanders-Bush and Steranka, 1978) followed by long-term depletion (Miller et al., 1970), on retention of a one-trial passive avoidance task was the main focus of the investigation (Experiments 1–5). To increase the generalizability of the findings, performance by *p*-chloroamphetamine-treated rats in a radial arm maze (Experiment 6), the effects of lesions of the dorsal raphe nucleus on passive avoidance retention (Experiment 7), and dorsal raphe lesion effects on the memory-disrupting effects of *p*-chloroamphetamine (Experiment 8) were also examined.

2. Materials and methods

2.1. Subjects

The subjects were 392 young adult (90 to 120-day-old) male Sprague-Dawley rats purchased from Charles River Company (Wilmington, MA). The animals were housed in groups of three or four in suspended wire-mesh cages (40.6 × 25.4 × 17.8 cm) and were maintained on a 12:12 h light/dark cycle with ad libitum access to Purina rat chow and water except when noted. Behavioral testing was conducted during the light phase.

2.2. Apparatus

All experiments, except Experiment 6, used passive avoidance as the assessment instrument. Passive avoidance training and testing were done in a two-compartment black/white shuttle box (35 × 28 × 16 cm) with a guillotine door separation and a stainless steel grid floor through which scrambled electric foot shock could be administered. A radial maze, constructed of wood and painted black, was used in Experiment 6. The

maze consisted of eight 46.0 × 8.7 × 8.5 cm arms and a 25.8 cm wide central platform. A small circular white receptacle (2.5 cm wide, 1 cm deep) was located at the end of each arm.

2.3. General procedure

2.3.1. Passive avoidance

The rats were initially placed in the white compartment of the shuttle box for 60 s after which time the guillotine door separating the two compartments was raised. Once the animals crossed into the black compartment (typically within 30 s) the guillotine door was lowered and a mild, short-duration foot shock was administered (see specific experiments for shock intensities and durations). The subjects remained on the black side for 60 s after shock termination. Retention was assessed at various intervals following training by placing the animal back on the white side with free access to the black compartment after 60 s. The latency to cross (with three paws) into the previously shocked black compartment served as the measure of retention. The apparatus was wiped clean with a deodorizer between the training and test sessions of individual animals.

2.3.2. Drug preparation

Solutions containing *p*-chloroamphetamine HCl (Sigma Chemical, St. Louis, MO) were mixed freshly daily with saline in light-tight bottles and injected i.p. All doses refer to the salt weight of the drug.

2.3.3. Biogenic aminergic neurochemistry

The animals were killed by rapid decapitation and dissections were performed on a 0°C cold plate. Tissue samples were frozen at −80°C until assayed. Measurement of biogenic amines and their metabolites was performed by high performance liquid chromatography with electrochemical detection (HPLC-EC) using a modification of the methods of Maruyama et al. (1980). Biogenic amines and metabolites were extracted from tissue samples with perchloric acid to precipitate protein, and 50-μl aliquots of the supernatant were injected into the HPLC-EC system (BioAnalytical Systems). The concentrations of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and norepinephrine were expressed as μg/g wet tissue weight. Percent depletions were calculated by comparing treated groups to the control group.

2.4. Methods for individual experiments

2.4.1. Experiment 1: dose-response curve for p-chloroamphetamine (passive avoidance and neurochemistry)

Different doses of *p*-chloroamphetamine (0.0, 0.5, 1.0, 2.5 mg/kg) were administered 30 min prior to passive avoidance training ($n = 5$ per group) within two replications using different foot shock parameters (Replication 1: 0.6 mA/2 s; Replication 2: 0.8 mA/2 s). Retention in both replications was assessed 72 h after training. The animals were killed within 4 h of testing and their frontal cortices were dissected out and stored at −80°C until assayed.

2.4.2. Experiment 2: time course for p-chloroamphetamine effect (passive avoidance and neurochemistry)

The animals were injected with either saline or 2.5 mg/kg *p*-chloroamphetamine (the dose which produced the greatest impairment in Experiment 1) at various intervals (15 min, 30 min, 45 min, 120 min, 24 h, 1 week) prior to passive avoidance training (1.0 mA/2 s) and were tested for retention 24 h later ($n = 10$–12 per group).

The effects of 2.5 mg/kg of *p*-chloroamphetamine injected at various intervals prior to killing on frontal cortical aminergic concentrations were examined in a concurrent study. The animals were injected with *p*-chloroamphetamine either 15, 30, 45, 60, or 120 min prior to killing ($n = 6$ per group). One saline-injected animal was also killed at each of the five intervals. In addition, another saline-injected animal was killed immediately after being injected. These six saline-injected animals served as the control group.

2.4.3. Experiment 3: effect of pre-training p-chloroamphetamine on short-term passive avoidance retention

Experiment 3 was conducted to determine whether pre-training administration of *p*-chloroamphetamine affected short-term passive avoidance retention performance. In this experiment animals were injected with either saline or 2.5 mg/kg *p*-chloroamphetamine 30 min prior to training (1.0 mA/2 s) and tested either 5 min, 1 h, or 48 h post-training ($n = 10$ per group).

2.4.4. Experiment 4: effect of pre-training and pre-test p-chloroamphetamine on long-term passive avoidance retention

To rule out the possibility that pre-training administration of *p*-chloroamphetamine was producing passive avoidance retention deficits via a state-dependent mechanism (Overton, 1978), Experiment 4 employed the typical 2 × 2 state-dependent design. Either saline or *p*-chloroamphetamine was administered 30 min prior to both the training (1.0 mA/2 s) and test sessions. This method yielded four treatment groups with drug conditions at training and testing either matched or mismatched ($n = 6$ per group). Retention was assessed 48 h following training.

2.4.5. Experiment 5: effect of pre-training vs. post-training p-chloroamphetamine on long-term passive avoidance retention

To evaluate whether *p*-chloroamphetamine influenced post-training consolidation processes, the effects of pre- vs. post-training *p*-chloroamphetamine administration on passive avoidance retention were evaluated in Experiment 5. Either saline or *p*-chloroamphetamine (2.5 mg/kg) was injected either 30 min prior to, or immediately after, passive avoidance training (1.0 mA/2 s) with testing for all subjects occurring 72 h after training ($n = 8–12$ per group).

2.4.6. Experiment 6: effect of pre-test p-chloroamphetamine on long-term radial arm maze retention

The consequences of serotonergic release for retrieval processes were examined further by assessing *p*-chloroamphetamine's effects on performance of a well-learned radial arm maze task ($n = 7$). Following a pre-training phase during which the receptacles in all eight arms of the maze were baited with water, 23-h water-deprived animals were required to drink water from receptacles in four of the eight arms during testing. Throughout the test phase, the same four receptacles were consistently baited. The animals were tested with drug for 15 days (5 days/week). Starting on day 16, through to day 24, three animals were injected with *p*-chloroamphetamine (2.5 mg/kg) 30 min prior to each daily session while the remaining four animals received saline. On day 25, the drug injections were reversed, with animals previously injected with saline given *p*-chloroamphetamine, and vice versa. Testing continued under these conditions until the end of the experiment (day 29). The numbers of working and reference memory errors, along with the total number of errors (working + reference), were recorded. A working memory error was defined as the second and subsequent visits to a (1) previously baited but now emptied arm, (2) never baited arm, or (3) baited arm without drinking. The first visit to an unbaited arm or the first visit to a baited arm without drinking constituted a reference memory error. Entrance with at least three paws into an arm was considered an arm entry, while a correct response also required that water was drunk from the arm's receptacle. The maze was wiped clean with a deodorizer between the test sessions with individual animals.

2.4.7. Experiment 7: effect of dorsal raphe lesions on long-term passive avoidance retention

The effect of global serotonergic depletion on long-term retention of passive avoidance was examined in this experiment. Serotonergic depletion was accomplished by stereotaxic infusion of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 15 μg/μl, 0.2% ascorbic acid solution) into the dorsal raphe nucleus. The animals were initially injected (i.p.) with 20 mg/kg per ml desmethylimipramine (Sigma) 20 min before surgical anesthesia (Björklund et al., 1975). Following anesthesia (60 mg/kg per ml ketamine HCl, i.m., Ketalar, Parke-Davis, Morris Plains, NJ; and 21 mg/kg per ml sodium pentobarbital, i.p., Sigma), each rat was positioned in a Baltimore stereotaxic instrument with the upper incisor bar set at 0.0. Infusions of 1 μl of 5,7-DHT into the dorsal raphe nucleus were made through a 33-gauge cannula positioned 30° off the vertical plane at stereotaxic coordinates +1.5 mm from the interaural line, + or −4.0 mm from the midline (right and left side approaches used approximately equally often) and −8.0 mm from the level of the skull. Sham-operated animals underwent the same surgical procedure except that the cannula was lowered −6.0 mm below the skull and the neurotoxin was not infused. One week following surgery, the rats were trained on passive avoidance (0.8 mA/2 s) and tested 72 h later. On the day after testing, all animals were killed and their cortices (frontal and occipital) and hippocampi were dissected out and stored at −80°C until assayed for 5-HT, 5-HIAA, dopamine, DOPAC, HVA, and norepinehprine.

2.4.8. Experiment 8: effect of p-chloroamphetamine administration to rats with dorsal raphe lesions on long-term retention of passive avoidance

The lesion procedures were identical to those described for Experiment 7. Twenty rats received lesions of the dorsal raphe, while another 20 rats received sham lesions. Behavioral assessment began 2 weeks after the lesions were made. The sham- and dorsal raphe-lesioned groups were further subdivided. Ten rats from each lesion group were pretreated with 2.5 mg/kg *p*-chloroamphetamine 30 min prior to passive avoidance training, while the remaining rats received saline injections. The passive avoidance training and testing parameters and procedures were identical to those described for Experiment 7. All rats were killed 1 week after the retention test and their frontal cortices were kept frozen until assayed for serotonin and 5-HIAA.

Table 1
Mean (±S.E.M.) passive avoidance training and 72-h retention latencies (s) of animals treated with various doses of *p*-chloroamphetamine 30 min prior to training in Experiment 1

Dose	Training latency	Retention latency
0.0 mg/kg	20 (±10)	437 (±99)
0.5 mg/kg	12 (± 3)	290 (±99)
1.0 mg/kg	20 (± 5)	121 (±26) [a]
2.5 mg/kg	12 (± 2)	75 (±28) [b]

[a] $P < 0.05$, [b] $P < 0.01$ vs. 0.0 mg/kg group.

Table 2
Mean percent depletions of biogenic amines and their metabolites in the frontal cortex following various doses of *p*-chloroamphetamine injected 72–76 h prior to killing in Experiment 1

Dose	5-HT	5-HIAA	DA	DOPAC	HVA	NE
0.0 mg/kg	0.0	0.0	0.0	0.0	0.0	0.0
0.5 mg/kg	+2.8	+10.5	−20.0	−9.1	−15.4	−6.2
1.0 mg/kg	+3.2	−4.3	0.0	0.0	+15.4	−8.9
2.5 mg/kg	−44.8 [b]	−31.1 [a]	+15.0	−4.5	+12.8	+6.7

Three animals from each replication served in each dose group. Negative values represent depletions. Depletions are relative to 0.0 mg/kg group (5-HT = serotonin; 5-HIAA = 5-hydroxyindoleacetic acid; DA = dopamine; DOPAC = dihydroxyphenylacetic acid; HVA = homovanillic acid; NE = norepinephrine; [a] $P < 0.10$; [b] $P < 0.001$ vs. 0.0 mg/kg group.

2.5. Statistical analysis

All data were analyzed with parametric analyses of variance (ANOVA) followed by pairwise tests (Experiments 1–6 and 8) or independent Student's *t*-tests (Experiment 7), as deemed appropriate for the design. Newman-Keuls post-hoc tests were used when all pairwise comparisons were to be made while orthogonal planned *t*-tests were used when specific a priori pairwise comparisons were to be made (i.e., a *p*-chloroamphetamine-treated group vs. its saline control group).

3. Results

3.1. Experiment 1: dose response curve for p-chloroamphetamine (passive avoidance and neurochemistry)

Because initial ANOVA indicated no significant effect of replication on training or test latencies ($P > 0.20$), data from the two replications were combined (Table 1).

A one-way ANOVA of the retention test results revealed a significant main effect of DOSE [$F(3,32) = 4.90$, $P < 0.01$]. Significant differences between saline-injected animals and those receiving the 1.0 ($P < 0.05$) and 2.5 ($P < 0.01$) mg/kg doses were confirmed with Newman-Keuls tests. In contrast, *p*-chloroamphetamine administration had no effect on training cross-through latencies ($P > 0.20$).

Frontal cortical levels of the biogenic amines and their metabolites are presented in Table 2. Serotonin was significantly depleted ($P < 0.01$) and 5-HIAA was marginally ($P < 0.10$) depleted only in those animals treated with 2.5 mg/kg *p*-chloroamphetamine. Levels of norepinehprine, dopamine and their metabolites were not significantly affected by any of the doses of *p*-chloroamphetamine ($P > 0.20$).

3.2. Experiment 2: time course for p-chloroamphetamine (passive avoidance and neurochemistry)

No significant effects were detected when training latencies were examined. In contrast, retention latencies (Fig. 1) were different between the groups, as reflected by a significant effect of DRUG [$F(1,112) = 9.22$, $P < 0.01$].

Orthogonal *t*-tests comparing *p*-chloroamphetamine-treated groups with their saline counterparts indicated statistically significant lower latencies at the 15-min, 30-min and 2-h injection to training intervals ($P < 0.05$).

Mean percent depletions of amines and their metabolites derived from the associated neurochemistry study are presented in Table 3.

Table 3
Mean percent depletion of biogenic amines and their metabolites in the frontal cortex following 2.5 mg/kg *p*-chloroamphetamine injected at various intervals prior to killing in Experiment 2

Injection to killing interval	5-HT	5-HIAA	DA	DOPAC	HVA	NE
15 min	+10.4	−22.0 [b]	−2.4	−10.0	−23.1	+6.6
30 min	+7.8	−11.9	+4.9	−34.5	−13.5	+15.8
45 min	−3.0	−9.9	+37.8	−51.7	−12.5	+14.5
60 min	−31.1 [a]	−14.7 [a]	+19.5	−55.2	−20.2	+6.1
120 min	−51.9 [c]	−27.8 [c]	+11.0	−65.5 [a]	−36.5 [a]	−3.9

Negative values represent depletions. Depletions are relative to the saline-injected control group. See Table 2 for abbreviations. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. saline-injected group.

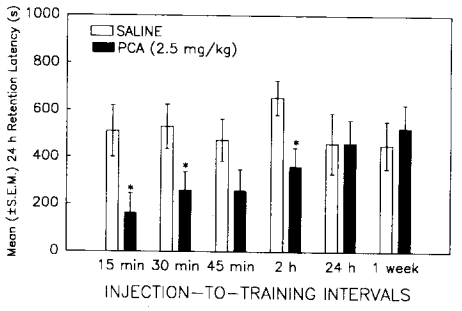

Fig. 1. Mean (±S.E.M.) 24-h passive avoidance retention latencies for saline- and *p*-chloroamphetamine (PCA, 2.5 mg/kg)-treated rats in Experiment 2. Injections were administered at various times prior to the training trial. Significant differences on testing between *p*-chloroamphetamine-injected animals and their respective saline control group existed at the 15-min, 30-min, and 2-h injection-to-training intervals (* $P \leq 0.05$)

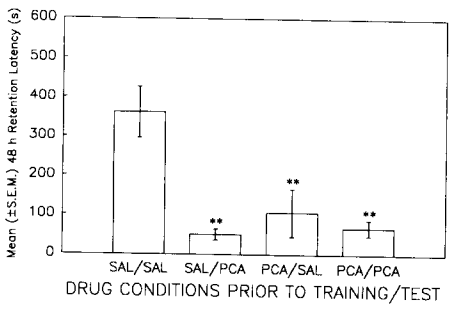

Fig. 3. Mean (±S.E.M.) 48-h passive avoidance retention latencies for saline (SAL)- and *p*-chloroamphetamine (PCA, 2.5 mg/kg)-treated subjects in Experiment 4. Injections were made 30 min prior to both the training and the test sessions. Significant differences existed between animals that received saline prior to both training and testing (SAL/SAL) and the three other groups (* * $P < 0.01$).

Separate one-way ANOVA performed for each amine or metabolite revealed differences between the groups for 5-HT, 5-HIAA, DOPAC and HVA levels [all $F(5,30) > 2.99$, all $P < 0.05$]. Subsequent Newman-Keuls tests indicated lower 5-HT and 5-HIAA concentrations in the cortices of rats killed 60 min ($P < 0.05$) and 120 min ($P < 0.001$) after *p*-chloroamphetamine injection. 5-HIAA was also significantly depleted 15 min following *p*-chloroamphetamine administration ($P < 0.01$). Significant differences in the concentrations of DOPAC and HVA between treated and saline injected animals were observed only at the longest (120 min) *p*-chloroamphetamine injection to killing interval ($P < 0.05$).

3.3. Experiment 3: effects of pretraining p-chloroamphetamine on short-term passive avoidance retention

p-Chloroamphetamine administration did not affect training latencies ($P > 0.10$), but did alter test latencies [$F(2,54) = 5.54$, $P < 0.01$] (see Fig. 2).

Orthogonal *t*-tests confirmed that, relative to their saline-treated control group, *p*-chloroamphetamine-injected animals tested 48 h after training exhibited significantly shorter test latencies ($P = 0.05$). *p*-Chloroamphetamine-injected rats tested at either 5 min or 1 h following training showed retention scores comparable to those of the controls ($P > 0.10$).

3.4. Experiment 4: effects of pre-training and pre-test p-chloroamphetamine on long-term passive avoidance retention

As in all previous experiments, pre-training injections of *p*-chloroamphetamine did not alter training cross-through latencies ($P > 0.20$). ANOVA applied to the retention data indicated a statistically significant interaction between the PRETRAIN and PRETEST injection variables [$F(1,20) = 7.35$, $P < 0.05$] (see Fig. 3).

Pairwise tests (Newman-Keuls) confirmed group differences between SAL/SAL animals and the other three groups ($P < 0.01$).

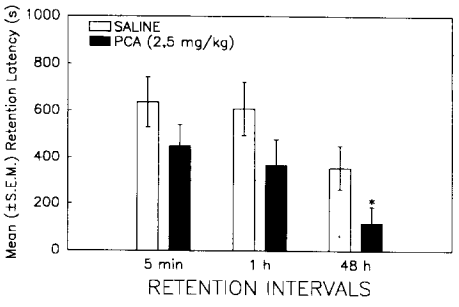

Fig. 2. Mean (±S.E.M.) passive avoidance retention latencies for saline- and *p*-chloroamphetamine (PCA, 2.5 mg/kg)-treated subjects in Experiment 3. All animals were injected 30 min prior to training and were tested at one of three intervals. A significant difference between *p*-chloroamphetamine- and saline-injected groups existed only at the 48-h retention interval (* $P = 0.05$).

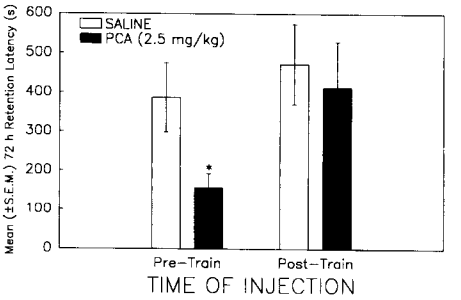

Fig. 4. Mean (±S.E.M.) 72-h passive avoidance retention latencies for saline- and *p*-chloroamphetamine (PCA, 2.5 mg/kg)-treated subjects in Experiment 5. Injections were made either 30 min prior to, or immediately after, training. The only statistically significant effect detected was when the performance of the pre-training *p*-chloroamphetamine group was compared to the performance of its saline-injected control group (* $P < 0.05$).

Table 4
Mean (±S.E.M.) working memory (WM), reference memory (RM), and total errors committed by saline (SAL)- and *p*-chloroamphetamine (PCA, 2.5 mg/kg)-injected rats trained on an 8-arm radial maze task in Experiment 6

Drug day	Drug	Mean (±S.E.M.) error		
		WM	RM	Total
Day before	(SAL)	1.3 (±0.6)	1.7 (±0.4)	3.0 (±0.9)
	(PCA)	0.9 (±0.5)	1.7 (±0.5)	2.6 (±0.8)
First	SAL	0.7 (±0.3)	2.3 (±0.4)	3.0 (±0.5)
	PCA	5.4 [b] (±0.9)	4.1 [a] (±0.4)	9.6 [b] (±1.0)
Second	SAL	1.7 (±0.6)	2.3 (±0.5)	4.0 (±1.0)
	PCA	1.7 (±0.6)	3.3 (±0.4)	5.0 (±0.9)
Third	SAL	1.4 (±0.6)	2.4 (±0.5)	3.9 (±0.9)
	PCA	1.0 (±0.4)	2.0 (±0.4)	3.0 (±0.8)

All comparisons are within the same type of error. (SAL) and (PCA) designate the drugs that were to be administered on subsequent days ([a] $P < 0.05$ vs. all other groups except second/SAL and second/PCA groups; [b] $P < 0.01$ vs. all other groups).

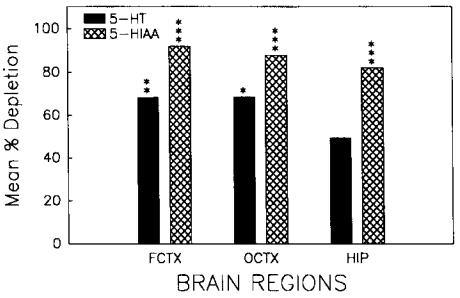

Fig. 5. Mean percent depletion of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex (FCTX), occipital cortex (OCTX) and hippocampus (HIP) of animals 1 week following 5,7-DHT-induced lesions of the dorsal raphe nucleus in Experiment 7. Percent depletions are relative to sham-operated subjects. Significant depletions of both 5-HT and 5-HIAA were noted in all brain regions except for 5-HT in the hippocampus (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

3.5. Experiment 5: effects of pre-training vs. post-training p-chloroamphetamine on long-term passive avoidance retention

As seen in Fig. 4, a main effect of PRETRAIN vs. POSTTRAIN administration was detected when retention scores were analyzed [$F(1,35) = 4.10$, $P = 0.05$].

Pre-training, but not post-training, administration of *p*-chloroamphetamine yielded lower test scores relative to saline-injected control animals (orthogonal *t*-test, $P < 0.05$).

3.6. Experiment 6: effects of pretest p-chloroamphetamine on long-term radial arm maze retention

p-Chloroamphetamine administration affected retrieval of a well-learned radial arm maze task as reflected by increased working memory, reference memory, and total errors (all $F > 4.63$, all $P < 0.02$) (see Table 4).

Significant increases in all three types of errors were detected on the first day of drug administration. However, on subsequent days, *p*-chloroamphetamine's deleterious effects waned. The performance of drug-treated animals was not only comparable to that of saline controls, but returned to its baseline (i.e., 'day before'). As seen in Table 4, *p*-chloroamphetamine disrupted task performance and increased all three types of errors only on the first day of its administration (working memory and total errors committed on the first day vs. all other comparisons: all $P < 0.01$; reference memory errors committed on the first day vs. all other comparisons except second day/SAL and second day/PCA: all $P < 0.05$; Newman-Keuls). Injections on subsequent days failed to disrupt task performance (all $P > 0.20$). Error rates on the third day of *p*-chloroamphetamine were almost identical to what they had been prior to drug administration and thus had returned to their baseline levels (all $P > 0.20$).

Table 5
Mean (±S.E.M.) passive avoidance training and 72-h retention latencies of animals prepared with either sham or dorsal raphe nucleus (DR) lesions in Experiment 7

Group	Training latency	Retention latency
Sham	33 (±10)	222 (±51)
DR	24 (±4)	216 (±69)

3.7. Experiment 7: effects of dorsal raphe lesions on long-term passive avoidance retention

Lesions of the dorsal raphe nucleus had no effect on passive avoidance training or test performances as seen in Table 5 (both $P > 0.20$).

Such lesions, however, significantly depleted sero-

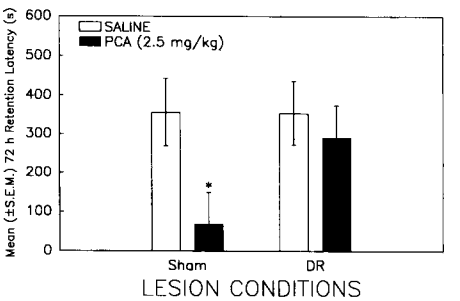

Fig. 6. Mean (±S.E.M.) 72-h passive avoidance retention latencies for sham- and dorsal raphe (DR)-lesioned animals treated either with saline or *p*-chloroamphetamine (PCA, 2.5 mg/kg) 30 min prior to training in Experiment 8. Only sham-lesioned animals treated with *p*-chloroamphetamine were impaired. Dorsal raphe lesions alone or dorsal raphe lesions combined with *p*-chloroamphetamine administration failed to alter test scores (* $P < 0.05$).

Table 6
Mean percent depletions of 5-HT and 5-HIAA within the frontal cortex of sham- and dorsal raphe nucleus (DR)-lesioned animals treated with either saline (SAL) or 2.5 mg/kg *p*-chloroamphetamine (PCA) in Experiment 8

Group	5-HT	5-HIAA
Sham/SAL	0.0	0.0
Sham/PCA	−48.5 [a]	−41.1 [a]
DR/SAL	−83.8 [b]	−74.3 [b]
DR/PCA	−87.4 [b]	−64.2 [b]

Negative values represent depletions. Depletions are relative to the sham/SAL group. See Table 2 for abbreviations. [a] $P < 0.05$, [b] $P < 0.01$ vs. sham/SAL group).

tonergic markers in the cerebral cortex and hippocampus (see Fig. 5).

Statistically significant depletions of 5-HT (both $P < 0.05$) and 5-HIAA (both $P < 0.001$) in the frontal and occipital cortices were observed, and significant depletion of 5-HIAA was detected in the hippocampus ($P < 0.001$). Marked, but statistically non-significant, depletion of 5-HT (49.6%) in the hippocampus was also detected. None of the other amines or metabolites examined was affected by the lesion procedure (all $P > 0.20$).

3.8. Experiment 8: effects of p-chloroamphetamine administration to rats with dorsal raphe lesions on long-term retention of passive avoidance

Lesions of the dorsal raphe nucleus failed to affect the 72-h retention of passive avoidance, whereas the pre-training administration of *p*-chloroamphetamine produced a significant impairment of retention test performance in sham-lesioned rats [$F(3,36) = 3.2$, $P < 0.05$]. As illustrated in Fig. 6, the only group which was adversely affected by *p*-chloroamphetamine treatment was the sham-operated group. Lesions of the dorsal raphe nucleus protected against the performance deficit induced by *p*-chloroamphetamine ($P < 0.05$).

5,7-DHT lesions of the dorsal raphe nucleus depleted frontal cortical 5-HT levels by an average of 83.8% ($P < 0.01$), whereas 5-HIAA levels were reduced by 74.3% ($P < 0.01$) (see Table 6). Although the administration of *p*-chloroamphetamine did not increase the depletion of serotonergic markers in dorsal raphe-lesioned rats ($P > 0.05$), it did reduce the levels of 5-HT and 5-HIAA in sham-lesioned animals (mean depletions of 48.5% and 41.1%, respectively, $P < 0.05$).

4. Discussion

The effects of serotonin release and depletion in rats trained in two tests of memory were compared and contrasted in eight experiments. Together, the results indicated that excessive release, but not depletion, of serotonin impaired retention performance of both the passive avoidance and radial arm maze tasks. For instance, depletion of serotonin in the frontal cortex and hippocampus, produced either by the long-term residual effects of *p*-chloroamphetamine (i.e., 24-h and 1-week injection-to-training intervals in Experiments 2) or by neurotoxic lesions of the dorsal raphe nucleus (Experiments 7 and 8), failed to affect retention scores despite reducing the levels of 5-HT and 5-HIAA by approximately 45-85% at the time of task acquisition. In contrast, 1 mg/kg *p*-chloroamphetamine administered shortly prior to training did impair later retention while leaving the concentration of 5-HT and 5-HIAA unaltered (Experiment 1). Experiment 8 demonstrated that the effects of pretraining *p*-chloroamphetamine could be ameliorated by preexisting lesions of the dorsal raphe nucleus, suggesting that when the releasable pool of serotonin is depleted by dorsal raphe lesions, the amnesic properties of *p*-chloroamphetamine are blocked.

Serotonin release appeared to have either affected the ability to retrieve what was learned or disrupted long-term consolidation, rather than impaired acquisition processes themselves. Passive avoidance deficits were observed only when subjects were injected with *p*-chloroamphetamine shortly prior to training and assessed for retention more than 1 h after training had ended. In contrast, post-training injections (Experiment 5) or injections administered more than 2 h before training (Experiment 2) did not yield deficits, nor were deficits observed when *p*-chloroamphetamine was administered 30 min prior to training and the animals were tested less than 1 h after training (Experiment 3), an effect consistent with previously reported results (Ögren, 1986b). Interestingly, like the time-dependent nature of *p*-chloroamphetamine effects, time-dependent effects of 5-HT antagonists have also been reported with pre-training administration yielding impairment and post-training administration producing improvement in rats trained on a lick suppression task (Altman and Normile, 1987). Collectively, the present results suggest that pharmacologically induced increases in the release of serotonin at, or very shortly after, training impairs retention performance. These data are consistent with the results of experiments exploring the mnemonic effects of various serotonergic receptor agonists, in that 5-HT agonists in general have been reported to impair learning and memory processes in animals tested with a variety of methods (Altman and Normile, 1988).

An alternative interpretation of the present passive avoidance data which focuses on state-dependent retention processes (Overton, 1978) was rejected based on data derived from Experiment 4. The effects of *p*-chloroamphetamine on passive avoidance performance were not related to a mismatch of internal

states since injections of *p*-chloroamphetamine prior to both the training and the test sessions failed to restore performance to control levels. Other possible non-cognitive explanations related to the drug's potential effects on motor ability or pain sensitivity were likewise rejected. Treated animals had unimpaired ambulatory behavior as their cross-through latencies on training throughout the study were consistently comparable to those of the controls. Injections of *p*-chloroamphetamine also failed to interfere with foot shock sensitivity as illustrated by the results from an ancillary study (data not presented) showing clearly that *p*-chloroamphetamine- and saline-treated animals exhibited similar flinch/jump thresholds, a finding consistent with the literature (cf., Le Bars, 1988). Moreover, additional data derived from Experiment 3 confirmed that foot shock sensitivity was not affected. Proficient retention performances by *p*-chloroamphetamine-treated animals were seen at the two shortest training-to-test intervals. The fact that these drug-treated rats exhibited proficient retention indicates that they were capable of detecting the training foot shock. Finally, it should be noted that Ögren (1986b) also similarly rejected these non-cognitive interpretations of *p*-chloroamphetamine's amnesic effects.

The results of Experiment 8 demonstrated that the *p*-chloroamphetamine effects on passive avoidance behavior, and by inference on radial arm maze performance, were dependent upon the existence of a releasable pool of serotonin. When these pools were depleted by dorsal raphe nucleus lesions, *p*-chloroamphetamine's effects on passive avoidance retention were blocked. It can also be concluded from these results that *p*-chloroamphetamine does not directly affect cognitive processes through its interaction with postsynaptic serotonergic receptors, nor are these processes affected indirectly by *p*-chloroamphetamine interacting with other, non-serotonergic, neurotransmitter systems. These conclusions are also supported by the effects of *p*-chloroamphetamine on radial arm maze performance (Experiment 7). In this experiment, *p*-chloroamphetamine disrupted performance after the first injection, but not after subsequent injections. This result is consistent with the hypothesis that *p*-chloroamphetamine exerts it mnemonic effects through the release of serotonin, since the releasable pool of serotonin would be expected to be significantly diminished after the first exposure to *p*-chloroamphetamine. Further support for the view that serotonergic depletion is without effect on learning and memory is provided by results of the studies by Riekkinen and his colleagues (Jäkälä et al., 1993; Riekkinen et al., 1992, 1993). Using the serotonin synthesis inhibitor, *p*-chlorophenylalanine, this group has consistently reported that learning and memory in rats trained on delayed non-matching to sample, passive avoidance, and Morris water maze were unaffected by a depletion of serotonin in the frontal cortex and hippocampus more extensive (in the 90% range) than those reported here (Jäkälä et al., 1993; Riekkinen et al., 1992, 1993). Moreover, similar to the findings from Experiments 7 and 8, Riekkinen's group has also observed no disruptive effects of dorsal raphe lesions on Morris water maze performance (Riekkinen et al., 1990). It is interesting to note that no impairment in performance was found following serotonergic depletion induced by the long-term effects of *p*-chloroamphetamine (2.5 mg/kg) in rats trained on a working memory version of the Morris water maze (Santucci et al., 1995). Together, these various findings suggest that reductions of serotonin do not directly produce defects in learning and memory.

An important point is the lack of specificity with regard to the effects of serotonin release on memory. Although the results indicated that acute release of serotonin produced a disruption in retention performance, the mechanism(s) by which this occurred remain(s) unresolved. It is well established that serotonin plays important roles in regulating or modulating a number of biological and behavioral activities such as sleep, feeding, mood, and aggression. Therefore, it is unlikely that an overall increase in serotonin would have specifically affected cognitive processes only. In other words, it is difficult to conclude that an increase in serotonin release *directly* affected memory. It is more reasonable to conclude that either the direct *or* indirect effects of serotonergic release were responsible for the memory impairment. In this vein, future studies with more specific pharmacological tools (e.g., receptor agonists or antagonists) and more specific anatomical localization are needed to better characterize the exact bases for the effects observed here.

Because a direct independent measure of serotonin release (e.g., in vivo microdialysis) was not applied, it can be argued that the present report gives no evidence for the view that serotonin release during training affects retention performance. Indeed, impairment of retention might simply reflect depletion of serotonin produced by *p*-chloroamphetamine's long-term neuropharmacological properties. This view could appear credible, especially as cortical depletions were noted 60–120 min after injections (see Table 3), a time course not totally inconsistent with the drug's amnesic effects. However, an interpretation which emphasizes decreased serotonin concentration becomes untenable when the data from Experiments 7 and 8 are considered. In these experiments, neurotoxic dorsal raphe lesions clearly failed to alter passive avoidance retention performance despite producing significant depletion of serotonin. Moreover, in Experiment 2, retention was unaffected when training occurred when the short-term release of serotonin induced by *p*-chloroamphetamine was no longer present but long-term

depletion of serotonin had developed. Furthermore, injections of 1 mg/kg in Experiment 1, which were administered 30 min prior to training, produced retention deficits but no decrease in cortical serotonin, a finding most consistent with the short-term temporal course (15–30 min) of *p*-chloroamphetamine-induced release of serotonin (Fuller, 1978; Ögren, 1985).

Since short-term decreases in both DOPAC and HVA were noted in Experiment 2 at the 2.5 mg/kg dose (see Table 3), alterations in dopaminergic metabolism at the time of acquisition might also have accounted for *p*-chloroamphetamine's amnesic effects. Although these changes were short-lived and are presumably related to *p*-chloroamphetamine's inhibitory effects on monoamine oxidase (Miller et al., 1970), their contribution to the retention-impairing effects of *p*-chloroamphetamine cannot be rejected based on the data available. However, a recent finding indicating improved learning in aged rats trained on a Morris water maze following monoamine oxidase-inhibition therapy with deprenyl (Brandeis et al., 1991) argues against the interpretation that decreases in dopaminergic metabolites played a significant role in *p*-chloroamphetamine's amnesia-producing effect in the present experiments. In order to reject fully a dopaminergic explanation, one simply needs to determine whether 1 mg/kg *p*-chloroamphetamine would produce short-term changes in DOPAC and HVA, since this dose too was effective to produce retention deficits. If dopaminergic parameters are not altered by this dose, short-term changes in dopamine metabolites cannot account for impairments in retention produced by *p*-chloroamphetamine.

Finally, a comment regarding the potential neurotoxicity of 2.5 mg/kg *p*-chloroamphetamine ought to be noted. This and similar doses have been shown to lack significant neurotoxic properties, especially within forebrain projecting raphe nuclei regions (Harvey et al., 1975; Miller et al., 1970). In contrast, much higher doses (e.g., 2×10 mg/kg) traditionally have been used to induce cell death of serotonin neurons (e.g., Normile et al., 1990). Therefore, it is unlikely that the present dose of *p*-chloroamphetamine produced significant neurotoxic effects. Rather, the depletion of serotonin reported in the present paper is most likely reversible (Miller et al., 1970) and reflects *p*-chloroamphetamine's well-known long-term neuropharmacological properties (Fuller, 1978).

In conclusion, it was noted in the Introduction that the results of studies on the modulation of learning and memory by serotonergic systems and serotonergic drugs have been inconsistent and, at times, confusing. Depending on the agent and mode used to affect the serotonergic system, enhancement, inhibition, or no effect has been reported. The present findings, however, indicated fairly clearly that excessive serotonergic release, and by implication, supra-normal stimulation of serotonergic postsynaptic receptors, produces impairment of mnemonic processes while forebrain serotonergic depletion has no direct effect. Although others have reached similar conclusions, the present report bases its inferences on results of studies with a consistent design and similar methods (e.g., passive avoidance, 2.5 mg/kg *p*-chloroamphetamine dose, etc.) used throughout. This may help to clarify some of the contradictions in the literature. With respect to the cognitive declines seen in Alzheimer's disease, the findings from the present investigation imply that the often reported serotonergic deficit found in this disease may not be directly responsible for memory impairments. However, this does not imply that serotonergic systems do not play an important role in Alzheimer's disease. It is entirely possible that serotonergic depletion modulates and interacts with other neurotransmitter systems, especially the cholinergic system (Altman et al., 1987; Richter-Levin and Segal, 1989; Riekkinen and Riekkinen, 1995; Riekkinen et al., 1990, 1992, 1993; Santucci et al., 1990, 1995). Therefore, in order to better understand the role of serotonin in Alzheimer's disease, future studies should aim to elucidate the interactions between serotonin and other neurotransmitter systems.

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