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p-Chloroamphetamine blocks physostigmine-induced memory enhancement in rats with unilateral nucleus basalis lesions

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Abstract

The present experiment examined whether *p*-chloroamphetamine (PCA), a serotonergic releasing/depleting agent, would block the memory-enhancing effect of physostigmine in rats with *N*-methyl-D-aspartic acid (NMDA)-induced unilateral lesions of the nucleus basalis of Meynert (uni-nbM). Six groups of subjects with uni-nbM lesions in addition to an isolated sham-operated control group were included. Subjects were trained and tested 72 h later on a one-trial passive avoidance task. Thirty minutes before training, rats with uni-nbM lesions were injected with either 1.0 or 5.0 mg/kg PCA or saline. Immediately after training, approximately half the subjects in each group were injected with either saline or 0.06 mg/kg physostigmine. Animals in the sham group received saline injections. Saline-injected animals with uni-nbM lesions performed poorly at test, a deficit that was reversed with physostigmine. Pretraining injections of PCA blocked physostigmine's memory-enhancing effect, although motor impairment during training may have contributed to decrements in test performance in animals injected with 5.0 mg/kg. Subjects were killed about 10 days later and their frontal cortices examined for choline acetyltransferase (ChAT). Results from the neurochemical analysis revealed that the lesion decreased ChAT levels and that the injection of 1.0 mg/kg PCA exaggerated this lesion-induced depletion. Implications for the interaction between acetylcholine and serotonin are discussed.
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1. Introduction

Evidence in support of serotonergic modulation of cholinergic processes exists at both the neural and behavioral levels. With regard to the former, previous results indicate that serotonin may regulate cholinergic activity within the cortex (Barnes et al., 1989; Bianchi et al., 1990; Consolo et al., 1996; Crespi et al., 1997; Diez-Ariza et al., 2002; Grazia Giovannini et al., 1998; Koyama et al., 1999; Maura et al., 1992; Riekkinen et al., 1990; Robinson, 1983; Santucci et al., 1990; Siniscalchi et al., 1990), hippocampus (Erb et al., 1997; Fujii et al., 1997; Izumi et al., 1994; Koyama et al., 1999; Maura et al., 1989; Nakai et al., 1998; Robinson, 1983; Shea et al., 1991; Wilkinson et al., 1994;), and striatum (Gillet et al., 1985; Jackson et al., 1988; Ladinsky et al., 1978; Rada et al., 1993). The exact nature of this neuromodulation, however, remains controversial but most likely depends on several factors including the receptor subtype studied and its pre- or postsynaptic localization, the pharmacological agent used, the brain region and its innervating pathway examined, and the neuroscientific methods and animal species used to investigate these systems. For example, stimulation of 5-HT_{1A} receptors in either the cortex (Consolo et al., 1996; Siniscalchi et al., 1990) or hippocampus (Erb et al., 1997; Fujii et al., 1997; Izumi et al., 1994; Nakai et al., 1998; Wilkinson et al., 1994) facilitates the release of acetylcholine, whereas activation of 5-HT_3 receptors in the cortex inhibits the release of acetylcholine (Barnes et al., 1989; Bianchi et al., 1990; Crespi et al., 1997; Diez-Ariza et al., 2002; Grazia Giovannini et al., 1998, Maura et al., 1992). These effects, however, have not been universally reported with investigators either failing to replicate them (Johnson et al., 1993; Grazia Giovannini et

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al., 1998; Maura et al., 1989) or reporting results in the opposite direction (Consolo et al., 1994).

As with neural regulation, serotonin interacts with acetylcholine to affect behavior (Lehman et al., 2000; Ögren et al., 1985a,b; Stewart et al., 1987), especially learning and memory (Cassel and Jeltsch, 1995; Decker and McGaugh, 1991; Steckler and Sahgal, 1995). One approach used by investigators to explore this interaction has been to examine the behavioral consequences of the serotonergic releaser/depleter *p*-chloroamphetamine (PCA) (Miller et al., 1970; Sanders-Bush and Steranka, 1978; Santucci et al., 1996) in cholinergically compromised animals. Results from such experiments have illustrated the effectiveness of depleting serotonin in aggravating the memory-impairing effects produced by blocking cholinergic nicotine (Riekkinen et al., 1992, 1993a,b) or muscarine (Riekkinen et al., 1991; Santucci et al., 1995b) receptors systemically, even though serotonergic depletions alone were without effect.

Additional evidence in support of a functional interaction between serotonin and acetylcholine is derived from a previous investigation from our laboratory that examined the memory modulating effects of increased release of serotonin induced by PCA in animals with lesions to a major cortically projecting cholinergic basal forebrain nucleus, the nucleus basalis of Meynert (nbM) (Santucci et al., 1990). We found that rats with bilateral nbM damage treated with 2.5 mg/kg PCA 30 min before passive avoidance training failed to respond to the memory-enhancing effects afforded by posttraining physostigmine—an effect most likely related to releasable pools of serotonin (Ögren, 1982), possibly from the dorsal raphe nucleus (Santucci et al., 1995a, 1996). This finding was in stark contrast to the test behavior of saline-injected lesion control subjects who were responsive to the memory-enhancing effects of posttraining administration of physostigmine. At test, these brain-damaged subjects exhibited proficient retention that was statistically indistinguishable from that of sham-operated control animals.

The data from our earlier study served as direct impetus for conducting the present experiment. Specifically, it was of interest to replicate and extend our previous findings by determining whether animals with NMDA-induced unilateral nbM lesions would similarly be unresponsive as animals with bilateral nbM damage to the memory-enhancing effects of physostigmine when injected with PCA before passive avoidance training. It was hypothesized that the release of serotonin engendered by a small dose of PCA (1.0 mg/kg) might be ineffective in blocking the memory-enhancing effect of physostigmine in animals with less extensive cholinergic damage. If true, we then further hypothesized that a larger dose of PCA (5.0 mg/kg) would be necessary to again block the memory-enhancing effect of physostigmine in such animals. Our goal was to provide insight into the relationship between extent of cholinergic damage and the dose of PCA needed to modulate memory processes.

2. Methods

2.1. Subjects

Seventy-three male adult (90- to 120-day-old) Sprague–Dawley rats obtained from Charles River (Wilmington, MA) were used. The animals were housed in groups of three or four in suspended wire-mesh cages (40.6 × 25.4 × 18.8 cm), and were maintained on a 12:12 h light/dark cycle with free access to Purina rat chow and water. Behavioral assessment was conducted during the light phase. The research was approved by the IACUC of the Department of Veteran Affairs Medical Center, Bronx, NY, and was conducted in accordance with the American Psychological Association's *Ethical Principles for the Treatment of Laboratory Animals* and the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

2.2. Surgery

Following ketamine HCL (60 mg/kg/ml im, Ketalar, Parke-Davis, Morris Plains, NJ) plus sodium pentobarbital (21 mg/kg/ml ip, Sigma Chemicals, St. Louis, MO) anesthesia, each rat was positioned in a Kopf stereotaxic instrument with the upper incisor bar set at 0. Unilateral neurotoxic lesions of the nbM (uni-nbM) were produced by slowly infusing by hand through a 33-gauge cannula 100 nmol/2 μl *N*-methyl-D-aspartic acid (NMDA; Sigma) administered over six stereotaxic sites [0.0, ± 2.8, and − 8.3 (0.6 μl), − 8.1 (0.2 μl) and − 7.8 (0.2 μl) mm ventral from skull; and − 0.7, ± 3.2, and − 8.0 (0.6 μl), − 7.8 (0.2 μl), and − 7.5 (0.2 μl) mm ventral from skull according to the stereotaxic atlas of Paxinos and Watson, 1986]. The cannula was left in place at each location site for 1–2 min so that diffusion could be achieved. Lesions of the left and right nbM were performed with approximately equal frequency. Sham lesions were conducted in an identical manner except the cannula was lowered − 6.0 mm from the skull and NMDA was not infused.

2.3. Drugs

Solutions containing *p*-chloroamphetamine HCL (PCA) and physostigmine salicylate (Physo) (Sigma) were mixed fresh daily with saline in light-tight bottles. Injections of PCA were performed intraperitoneally while subcutaneous administration was the method by which Physo was injected. Doses reported refer to the salt weight of each drug.

2.4. Apparatus

Assessment of passive avoidance was conducted 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. Individual hinged lids allowed

access to each compartment. A small 15-W bulb suspended over the white side was illuminated at all times and served as the sole source of ambient illumination. All training and testing was conducted in an isolated experimental room with white noise turned on to mask extraneous sounds.

2.5. Procedure

Approximately 3 to 4 weeks after surgery (mean = 24.3 ± 6.9 days) subjects were trained on a one-trial passive avoidance task. During training, each rat was 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 animal crossed into the black compartment the guillotine door was lowered and mild, short-duration foot shock (1.0 mA/2 s) was administered. The subject remained on the black side for 60 s after shock termination. Retention was assessed 72 h later 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 black compartment served as the dependent measure at both training and testing. The apparatus was wiped clean with a deodorizer between the training and test sessions of individual animals.

Thirty minutes before training subjects with uni-nbM lesions were injected intraperitoneally either with 0.0 (saline), 1.0, or 5.0 mg/kg PCA. Approximately half of the subjects in each of these groups were then injected subcutaneously with 0.06 mg/kg Physo immediately after training while the remaining half received injections of saline (0.00 mg/kg). All doses were administered in a volume of 1 ml/kg. Assigning subjects in this manner produced the following six treatment conditions: 0.0 mg/kg PCA + 0.00 mg/kg Physo ($n = 10$), 0.0 mg/kg PCA + 0.06 mg/kg Physo ($n = 10$), 1.0 mg/kg PCA + 0.00 mg/kg Physo ($n = 10$), 1.0 mg/kg PCA + 0.06 mg/kg Physo ($n = 11$), 5.0 mg/kg PCA + 0.00 mg/kg Physo ($n = 10$), and 5.0 mg/kg PCA + 0.06 mg/kg Physo ($n = 9$). Subjects in the sham-operated isolated control group ($n = 13$) received saline injections before and immediately after training. Animals were assessed on passive avoidance in two consecutive replication studies with approximately equal numbers of animals serving in each of the seven groups in both Replication 1 and Replication 2.

Within about 10 days after being tested, subjects from each replication study were killed by rapid decapitation and their frontal cortices dissected on a 0 °C cold plate (mean surgery-to-kill interval = 35.2 ± 7.6 days). Tissue samples were kept frozen at − 80 °C until assayed for choline acetyltransferase (ChAT) according to the methods of Fonnum (1975). Activity values for ChAT were reported in nanomoles acetylcholine per hour per milligram protein. The extent of enzymatic depletion in each animal was expressed as a percentage by applying the following formula: [(control value − lesion value) ÷ control value] × 100. Because extremely aberrantly low protein values from three animals in various unilateral nbM lesion conditions (one each from the 0.0 mg/kg PCA + 0.00 mg/kg Physo [low protein value on control side], 1.0 mg/kg PCA + 0.06 mg/kg Physo [low protein value on control side], and 5.0 mg/kg PCA + 0.06 mg/kg Physo [low protein value on lesion side] groups) from Replication 2 prevented the accurate assessment of the neurochemical marker, ChAT data from these subjects were not included in the statistical analyses. Another animal with a unilateral nbM lesion derived from the 5.0 mg/kg PCA + 0.00 mg/kg Physo group died before its brain was assayed. Eight of the 13 sham-operated control animals were randomly selected to be included in the neurochemical analysis.

2.6. Statistical analysis

All data were analyzed with a commercially available computer program (Statistica 6.0, StatSoft, ver. 6, 2001, Tulsa, OK). Because initial statistical analyses of the results indicated no statistically significant effect involving replication number for any of the dependent variables studied (all Ps >.05), the data from the two replications were combined and analyzed in aggregate form.

Factorial 3 (PCA) × 2 (Physo) between-subjects analyses of variance (ANOVAs) with an isolated control group were used to analyze the data from passive avoidance, while ChAT activity values were assessed with a factorial 3 (PCA) × 2 (Physo) × 2 (side) mixed design ANOVA with an isolated control group. A separate factorial 2 (PCA) × 2 (Phsyo) ANOVA with an isolated control group was used to analyze percent depletion scores. Finally, Fisher least significant difference tests were applied to detect pairwise differences, when appropriate.

3. Results

3.1. Passive avoidance

One sham-operated control animal froze when placed in the white compartment at training and subsequently failed to cross into the black compartment within 5 min. For this reason, this animal was discarded from the study and not included in the statistical analyses. Analysis of training latencies revealed only a significant main effect of PCA [$F(2,66) = 16.38$, $P < .00001$] (see Fig. 1). Relative to the other five groups of subjects, animals in the two groups treated with 5.0 mg/kg PCA exhibited sluggish and uncoordinated motor behavior. This resulted in these animals requiring significantly longer latencies to cross into the black compartment than all other subjects (all Ps ≤ .01). The mean training latencies associated with the two 5.0 mg/kg PCA groups did not differ from each other (both Ps>0.05). No other group differences were detected (all Ps>.05).

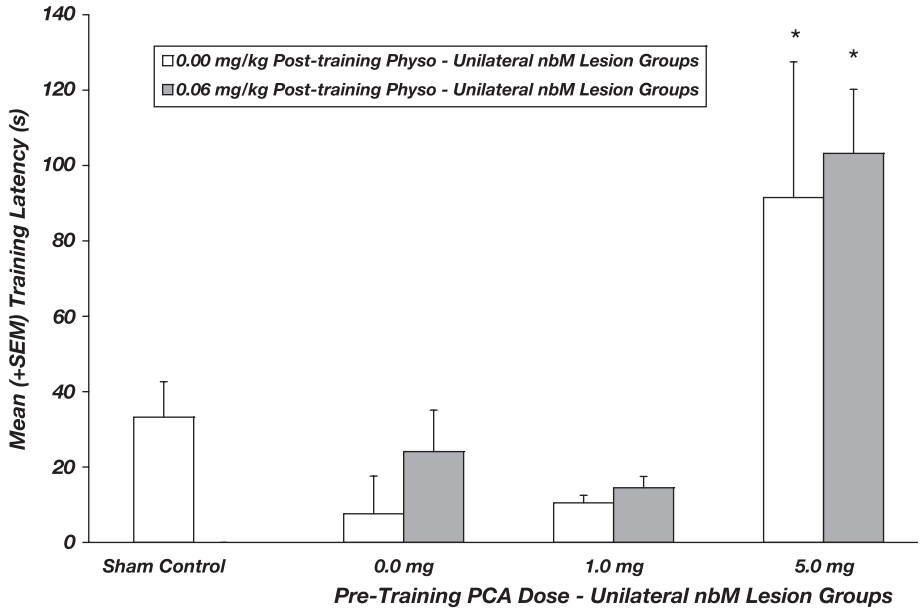

Fig. 1. Mean (+standard error of the mean, SEM) training cross-through latencies (s) for the seven groups of subjects. The mean latencies for both groups injected with 5.0 mg/kg PCA were significantly longer than the mean latencies associated with all other groups and did not differ from one another ($P > .05$). Note: at the time of training, animals had not yet been injected with Physo. ($^{*}P \leq .01$ vs. all other groups; nbM = nucleus basalis of Meynert; PCA = *p*-chloroamphetamine; Physo = physostigmine).

The results from the ANOVA used to analyze retention scores revealed a significant main effect of PCA [$F(2,66 = 10.31$, $P < .001$] (see Fig. 2). With one exception, all subjects with uni-nbM lesions exhibited shorter retention latencies relative to sham-operated controls (all Ps $< .0001$). The one exception was the group of subjects treated with 0.0 mg/kg PCA + 0.06 mg/kg Physo. The performance of this group did not differ statistically from that of sham-operated rats ($P > .05$) and was significantly better than that of all other groups of animals with uni-nbM lesions (all Ps $< .02$).

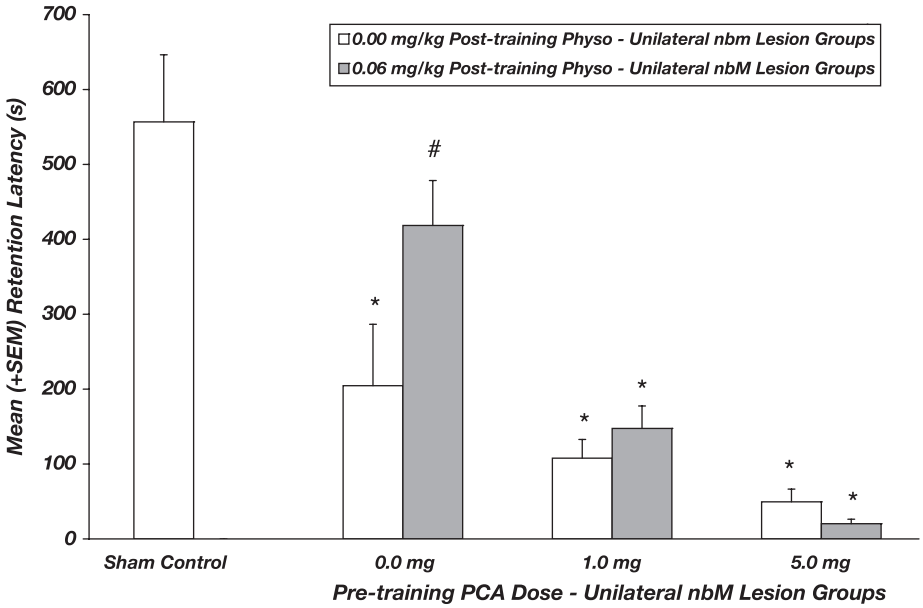

Fig. 2. Mean (+standard error of the mean, SEM) retention cross-through latencies (s) for the seven groups of subjects. With one exception, the mean latencies for all animals with unilateral nbM lesions were significantly shorter than the mean latency associated with sham control subjects. The one exception was the unilateral nbM lesion 0.0 mg/kg PCA + 0.06 Physo group whose mean latency did not differ from that of sham control animals ($P > .05$) and was significantly longer than the mean latencies for all other groups containing animals with unilateral nbM lesions. ($^{*}P < .0001$ vs. sham control; $^{\#}P < .02$ vs. all other groups containing animals with unilateral nbM lesions; nbM = nucleus basalis of Meynert; PCA = *p*-chloroamphetamine; Physo = physostigmine).

Table 1
Mean ChAT activity (nmol acetylcholine/h/mg protein) and percent depletion values

Lesion condition	PCA dose (mg/kg)	Physo dose (mg/kg)	Mean control value ± S.E.M.	Mean lesion value ± S.E.M.	Mean % depletion ± S.E.M.
Sham	0.0	0.00	45.4 ± 1.80	45.1 ± 2.35	− 3.4 ± 8.72
nbM	0.0	0.00	43.4 ± 2.33	38.3 ± 2.94	12.2 ± 3.65
	0.0	0.06	45.3 ± 2.01	36.1 ± 1.85	19.9 ± 3.76
	1.0	0.00	50.3 ± 2.43	34.5 ± 2.74	31.6 ± 4.26
	1.0	0.06	42.9 ± 1.67	37.4 ± 2.79	13.6 ± 4.15
	5.0	0.00	46.0 ± 1.64	37.8 ± 2.12	17.5 ± 4.31
	5.0	0.06	44.5 ± 1.63	34.1 ± 2.68	22.8 ± 6.51

"Mean % depletion" values are group averages based on the percent depletion achieved in each animal in that group. Percent depletions for individual animals were calculated by applying the following formula: [(control value − lesion value) ÷ control value] × 100. "Mean control" and "mean lesion" values are simple arithmetic group means based on raw ChAT values and were not used in calculating "mean % depletion" values. (nbM = nucleus basalis of Meynert; PCA = *p*-chloroamphetamine; Physo = physostigmine).

3.2. ChAT neurochemistry

Analysis of ChAT values revealed an expected main effect of side [$F(1,57) = 78.97$, $P < .000001$] and a significant three-way PCA × Physo × Side interaction [$F(2,57) = 6.42$, $P < .01$]. Relative to sham-operated control subjects, all animals with uni-nbM lesions exhibited significant unilateral depletion levels (all Ps $< .05$). The three-way interaction was related to the fact that subjects treated with 1.0 mg PCA + 0.00 mg Physo showed exaggerated ChAT depletions relative to the 0.0 mg PCA + 0.00 mg Physo group (i.e., the "lesion control" group) ($P < .01$). The only other significant pairwise difference was detected when the depletion values from the two 1.0 mg/kg PCA groups were compared with each other, with animals injected with 0.00 mg/kg Physo (i.e., saline) exhibiting a larger average depletion value (31.6% vs. 13.6%, $P < .02$) (Table 1).

4. Discussion

Similar to a number of previous reports from our laboratory (Haroutunian et al., 1985, 1990a,b; Santucci et al., 1989, 1990), results from the present experiment revealed that (1) NMDA-induced damage to the nbM produced significant passive avoidance retention deficits, (2) post-training administration of physostigmine reversed this memory impairment, and (3) pretraining injections of PCA blocked the memory-enhancing effect of physostigmine. The novel contribution of the present data was the demonstration of (1) NMDA lesion-induced retention impairment, and its reversibility by physostigmine, in animals with less extensive (i.e., unilateral) damage to the nbM and (2) the effectiveness of a relatively small PCA dose (1.0 mg/kg) in blocking physostigmine-induced facilitation of memory in animals prepared with unilateral nbM damage. These data are viewed as being consistent with the general perspective that (a) proficient retention is dependent on the bilateral integrity of the nbM and its cortical projections (Dekker et al., 1991) and (b) serotonin modulates cholinergic neurotransmission in such a way as to affect learning and memory (Cassel and Jeltsch, 1995; Decker and McGaugh, 1991, Steckler and Sahgal, 1995), even in the presence of cholinergic damage restricted to only one side of the brain.

In prior studies we have provided evidence that PCA in neurologically intact animals produces its effects on passive avoidance retention (Santucci et al., 1995a) and on working memory as assessed in a radial arm maze (Santucci et al., 1996) via releasable pools of serotonin from the dorsal raphe nucleus, rather than by depleting frontal cortical or hippocampal serotonin levels despite extensively diminished (45–85%) concentrations. By extension, we suggest that the basis for 1.0 mg/kg PCA's blockade of physostigmine-induced restoration of memory in animals with unilateral nbM damage observed here is related to releasable pools of serotonin, possibly from the dorsal raphe nucleus, rather than to PCA's well-known long-term serotonergic depleting effects within the nervous system. The fact that basal forebrain cells receive projections from serotonergic neurons derived from the dorsal raphe nucleus (Gasbarri et al., 1999) provides a neuroanatomical basis for our speculation. Pharmacologically, it is likely that a consequence of increased release of serotonin was to inhibit synaptic availability of acetylcholine (Santucci and Haroutunian, in press), possibly through presynaptic activation of 5-HT_{1A} (Rada et al., 1993), 5-HT_{1B} (Birthelmer et al., 2002; Feuerstein et al., 1996; Maura and Raiteri, 1986) and/or 5-HT_3 (Crespi et al., 1997) receptors. Activation of these serotonergic receptors and the subsequent diminished release of acetylcholine prevented physostigmine from exerting its memory-enhancing effect due to insufficient amounts of synaptic acetylcholine on which to work. In short, PCA-induced release of serotonin counteracted the beneficial effects of physostigmine on memory consolidation in animals with low levels of acetylcholine following lesions to the nbM. Because PCA retained its memory-modulating properties despite the presence of nbM damage restricted to only one hemisphere, one can conclude that cholinergically mediated cognitive processes are highly sensitive to serotonin's influence. Said differently, the integrity of the cholinergic forebrain system is necessary for cholinomimetic treatment to be effective in reversing the deleterious effects on memory produced by excessive serotonergic release (Matsuno et al., 1993, 1994). Once damage to the nbM occurs, regardless of its extensiveness, acetylcholinesterase inhibition treatment loses its memory-enhancing ability to reverse PCA-induced amnesia.

Mounting evidence suggesting a role for cholinergic processes from the nbM to the amygdala in learning, memory, and attention has been presented in the literature in recent years (Ingles et al., 1993; Mallet et al., 1995, McGaughy et al., 2002). Thus, the present findings may

involve the involvement of nbM-to-amygdaloid cholinergic projections. Previous reports have shown that in addition to producing cortical cholinergic depletions the excitotoxic lesion procedure also produces amygdalar cholinergic depletions (Beninger et al., 1994; Boegman et al., 1992). Accordingly, it can be argued that similar unintended damage was imparted in our subjects, and those cholinergic processes not within the cortex but rather within the amygdala mediated the memory impairing and memory-enhancing effects. The fact that intra-amygdala infusions of cholinergic agonists improve learning and memory (Barros et al., 2002; Dumery et al., 1988; Power et al., 2003; Schroeder and Packard, 2002) whereas infusions of cholinergic antagonists impair learning and memory (Barros et al., 2002) underscore the possible role played by cholinergic projections to the amygdala in the present study. Moreover, unilateral phthalic acid nbM lesions have been reported to produce memory impairment in a passive avoidance task that could be rescued with posttraining intra-amygdalar infusions of physostigmine (Power and McGaugh, 2002), and systemic cholinergic agonist treatments have been reported to require an intact amygdala to rescue passive avoidance retention behavior in rats with nbM lesions (Riekkinen et al., 1993a). Taken together with the data derived from PCA-treated subjects, it can effectively be argued herein that serotonergic innervation of the nbM from the dorsal raphe nucleus (Gasbarri et al., 1999) served to inhibit the already diminished release of acetylcholine provided to the amygdala from the nbM. It is entirely possible that cholinergic innervation of both the amygdala and the cortex provided by the nbM may interact in functionally important, as of yet undetermined, ways to modulate learning and memory processes. To illustrate, Power et al. (2002) have recently demonstrated the effectiveness of nbM lesions induced by the immunotoxin 192 IgG-saporin in blocking memory enhancement engendered by postacquisition intra-amygdala infusions of norepinephrine in animals trained on a passive avoidance task. Regardless of the exact neuroanatomical bases, the fact still remains that serotonergic neurotransmission serves to modulate cholinergic processes of the nbM involved in learning and memory.

A more mundane noncognitive alternative interpretation of the present results suggests that 1.0 mg/kg PCA altered sensory processes and thus interfered with the animal's ability to detect the training foot shock. Although there was no formal test of pain thresholds/sensitivity in the present study, we have previously reported that a higher dose of PCA (2.5 mg/kg) was without effect on foot shock sensitivity threshold levels in neurologically intact animals (Santucci et al., 1996). Moreover, in that report we observed PCA's deleterious effects on retention to be evident only at long retention intervals (i.e., >1 h); because proficient retention was noted when subjects were assessed at short retention intervals, the effect of PCA on retention performance in that study could not be attributed to a failure to experience foot shock at training. Finally, the consensus derived from a review of the literature (Le Bars, 1988) and other empirical investigations (Eide et al., 1988; Ögren et al., 1985a,b; Ögren and Johansson, 1985) similarly supports the view that PCA's effects on passive avoidance retention are not artifacts of altered sensory processes. In fact, there was at least one study cited in the review by Le Bars (1988) reporting PCA to have produced a decrease in pain threshold levels (i.e., increased pain sensitivity).

Similar to the 1.0 mg/kg PCA findings, the retention data derived from those animals with unilateral nbM lesions injected with 5.0 mg/kg PCA at training are also taken as evidence in support of the view that serotonin release interferes with the memory-enhancing effect of physostigmine. Animals injected with 5.0 mg/kg PCA were as unresponsive to cholinesterase inhibition treatment as were animals that had received injections of 1.0 mg/kg. It is noteworthy that Archer et al. (1981) also made a similar conclusion regarding the memory-impairing effects of this dose in animals trained on a shock-elicited fear-conditioning task. Unfortunately, however, attributing the effects of 5.0 mg/kg PCA to a modulation of cholinergic-mediated memory processes is clouded by the fact that injected animals showed significant motor impairments at training (e.g., sluggish motor behavior, uncoordinated limb movement, paws slipping through the grids) and had significantly longer training cross-through latencies. Although we have no reason to believe that a relatively high dose of PCA would be unable to block physostigmine's memory-enhancing effects in animals with unilateral lesions of the nbM, the existence of significant motor impairments in animals injected with 5.0 mg/kg of PCA preclude us from making a definitive conclusion regarding the ability of this dose to modulate memory processes in cholinergically damaged animals.

In addition to its modulatory effects on behavior, PCA also affected the degree to which ChAT enzyme concentrations within the frontal cortex were depleted following NMDA-induced lesions. As expected, neurotoxic-induced cholinergic damage yielded significantly lower levels of this enzyme within the cortex ipsilaterally to the lesion as has been reported innumerably by a number of investigators (see Dekker et al., 1991; Santucci and Haroutunian, in press, for reviews) including our laboratory (Haroutunian et al., 1985, 1990a,b; Santucci et al., 1990). What was notable was the demonstration that PCA exacerbated the degree to which ChAT was depleted, an effect interestingly observed previously in animals with bilateral NMDA-induced nbM damage (Santucci et al., 1990). Although we did not investigate the specific mechanism(s) that might account for this neurochemical outcome, one parsimonious possibility is that some cholinergic neurons in the nbM that are not directly destroyed by NMDA (e.g., those at the penumbra of the NMDA lesion sphere) are rendered vulnerable to PCA-induced degeneration. Injections of PCA subsequent to the lesion produced by NMDA may have led to further

degeneration in the nbM resulting in greater frontal cortical ChAT depletions. Remarkably, injections of physostigmine prevented PCA from aggravating NMDA-induced cortical enzymatic deficits. In effect, physostigmine appears to have protected vulnerable neurons against PCA damage. Although the exact mechanism of this outcome remains unknown, it may be related to a more general neuroprotective ability of cholinomimetics in nbM cells (Harkany et al., 2001), possibly linked to an increased expression of amyloid precursor protein following excitotoxic (NMDA-induced) damage (Harkany et al., 2000).

Finally, it must be acknowledged that the degree to which cortical ChAT was depleted in our animals was on the low end of the range reported in the literature. This was a planned outcome of the experimental design to avoid floor effects and allow us to determine whether serotonin would modulate cognitive processes in animals with mild cortical cholinergic deficits. As mentioned above, the fact that behavioral impairments were observed despite relatively low cortical ChAT depletions underscores the sensitivity of the nbM–cortical cholinergic pathway in mediating learning and memory processes. On the other hand, it might be argued that such minimal cortical cholinergic deficits are insufficient to produce passive avoidance retention deficits and, therefore, perturbations to other neurotransmitter systems (e.g., GABAergic) or coincident damage to other cholinergic pathways (e.g., nbM–amygdala, see above) must have been involved. In this regard, some investigators have used the purportedly cholinospecific immunotoxin 192 IgG-saporin to lesion cells of the nbM (Book et al., 1994; Pizzo et al., 1999; Schliebs et al., 1996). Using such an approach, targeted and relatively extensive damage to the nbM in the absence of learning and memory deficits have been reported (Baxter et al., 1995; Dornan et al., 1997; Galani et al., 2002; Wenk et al., 1994). This pattern of findings have led these researchers to conclude that cortically projecting cholinergic cells of the nbM do not contribute to learning and memory processes in a direct manner. At the same time, however, other investigators who have used 192 IgG-saporin to lesion cells of the nbM have concluded just the opposite. Using various different behavioral paradigms and experimental animals, researchers have provided evidence supportive of a role played by cholinergic basal forebrain cells in mediating learning and memory processes (Berger-Sweeney et al., 1994; Ridley et al., 1999; Zhang et al., 1996). Strongly supporting involvement of cortical mechanisms in the present study are the findings reported by Zhang et al. (1996) indicating that cell loss within the nbM correlated with the severity of passive avoidance impairments in rats with basal forebrain cholinergic lesions induced by 192 IgG-saporin. Thus, procedural differences, especially with respect to amount of training or overtraining, appear to be important factors in whether or not nbM lesion-induced deficits are observed.

Taken together, the behavioral and neurochemcial findings from the present experiment underscore the modulatory nature of serotonergic/cholinergic interactions. Additional studies investigating more closely the nature of this modulation in the present lesion model, especially experiments that examine the involvement of specific neuroanatomical sites and receptor subtypes, and the effects of other drug doses, will undoubtedly provide added insights into the specific nature of this interaction. Such studies will not only ultimately provide important information for understanding the neural foundations of learning and memory, but also will contribute to developing effective therapies for Alzheimer's disease and perhaps other dementias.

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