¹⁹²IgG-Saporin immunotoxin and ibotenic acid lesions of nucleus basalis and medial septum produce comparable deficits on delayed nonmatching to position in rats

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The recently developed immunotoxin, ¹⁹²IgG-Saporin (192-SAP), was compared with the standard excitotoxin, ibotenic acid, on two measures: (1) the extent of deficits on performance of a working memory task, delayed nonmatching-to-position (DNMTP), and (2) sensitivity to scopolamine on this task. Rats were extensively pretrained in an operant, spatial DNMTP memory task, then given combined site-specific lesions of the medial septum/diagonal band and nucleus basalis magnocellularis using either ibotenic acid (IBO) or low doses of the selective cholinergic immunotoxin 192-SAP. When compared with sham controls, both IBO and 192-SAP lesioned rats showed significant delay-independent reductions in DNMTP choice accuracy. Both 192-SAP and IBO lesioned rats showed increased sensitivity to a threshold dose of scopolamine, 0.15 mg/kg i.p., on DNMTP, as compared with sham-lesioned controls. When the rats were assessed at 18 weeks postlesioning, levels of choline acetyltransferase were depleted in the hippocampus in both IBO and 192-SAP lesioned groups. These findings suggest that 192-SAP, a cholinergically selective neurotoxin, is as effective as an excitotoxin when microinjected into cholinergic cell bodies of the basal forebrain, producing deficits in behavioral tasks that persist for several weeks.

One of the most consistent neurochemical alterations observed in Alzheimer's disease (AD) is the loss of markers for acetylcholine throughout the cortex and hippocampus, including the synthetic enzyme choline acetyltransferase (ChAT; Bowen, Smith, White, & Dawson, 1976; Davies & Maloney, 1976). This loss reflects degeneration of cholinergic neurons projecting from the area of the nucleus basalis and medial septum, and it may contribute to Alzheimer's dementia (Coyle, Price, & DeLong, 1982).

The evidence for cholinergic involvement in cognition is supported by a large literature describing amnesia induced in humans by cholinergic muscarinic receptor antagonists such as atropine and scopolamine (see Molchan et al., 1992). Performance of learning and memory paradigms in rodents and nonhuman primates is disrupted by cholinergic antagonists and lesions of the septohippocampal pathway (Hagan & Morris, 1988), further suggesting that the basal forebrain cholinergic systems play an important role in processes necessary for learning and memory.

In many animal studies, electrolytic or excitotoxic lesions of the cholinergic cell body regions of the nucleus basalis and medial septum-diagonal band have been used to model the cholinergic degeneration in AD (reviewed in Olton & Wenk, 1987). However, both methods also destroy noncholinergic cell bodies. Neuropharmacologically specific interpretations of behavioral deficits and unequivocal implication of basal forebrain cholinergic

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systems in learning and memory processes have proved impossible with these methods. A more selective cholinergic neurotoxin, ^{125}I gG-saporin (192-SAP), has recently been developed. Saporin, a ribosome-inactivating protein, is coupled to an antibody raised against the p75 NGF low-affinity receptor (Heckers et al., 1994; Wiley, 1992). In the basal forebrain region, this receptor is specifically expressed on cholinergic cell bodies, therefore allowing selective targeting.

The specific involvement of basal forebrain cholinergic neurons in learning and memory has been challenged by findings showing a lack of correspondence between decreases in markers of cholinergic destruction and behavioral deficits (reviewed by Dunnett, Everitt, & Robbins, 1991). In the present study, depletion of cholinergic markers is compared with behavioral deficits for the two types of lesions. The selective cholinergic toxin, 192-SAP, is compared with the standard nonspecific excitotoxin, ibotenic acid (IBO), injected into the nucleus basalis magnocellularis and medial septum, on baseline performance of an operant, delayed nonmatching-to-position (DNMTP) memory task. Further, sensitivity to a threshold dose of a muscarinic antagonist, scopolamine, on this task, was compared for the two lesion techniques. Scopolamine produces greater deficits in memory in persons with Alzheimer's disease than in age-matched controls (Molchan et al., 1992) and therefore might reveal deficits in lesioned animals when deficits are undetectable in baseline behavioral measures.

METHOD

Subjects

The subjects were 37 male, Sprague-Dawley rats, 120 days old at the beginning of the experiment. They were housed individually in stainless steel cages in a humidity- and temperature-controlled vivarium, and were maintained on a 12:12-h light:dark cycle (lights on at 7 a.m.). The subjects were allowed one half hour per day post-session access to water on weekday running days, and they were allowed unrestricted access to water on weekends when no behavioral testing was conducted. Body weights were taken daily to ensure that no subject fell below 85% of its free-drinking weight. No supplemental water was necessary to maintain 85% body weight for any subject during the months of regular behavioral training. All procedures were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the NIMH Animal Care and Use Committee.

Surgery

Stereotaxic surgery was conducted under chloral hydrate anesthesia (350 mg/kg i.p.). A complete lesion of the nucleus basalis magnocellularis was accomplished by performing multiple injections of IBO (Sigma, St. Louis) or 192-SAP (prepared in the laboratory of Ronald Wiley according to the protocol described in Wiley & Lappi, 1993, 1994) along the entire rostral-caudal extent. IBO is an extensively studied neurotoxin that destroys cell bodies but is nonspecific in its actions. It was chosen for comparison with 192-SAP for neurotoxic effects, and as a control for mechanical damage to tissue resulting from the multiple site injections. An additional control group, with sham surgery, underwent all surgical procedures except for the microinjections.

The subjects ($n = 20$) received a total of nine injections of IBO. Eight injections were designed to encompass the anterior-posterior

extent of the nucleus basalis ($0.1 \mu\text{g}/0.1 \mu\text{l}$ over 30 sec, bilaterally into four sites; coordinates from Paxinos & Watson, 1986): (1) -0.2 posterior to bregma, -6.0 ventral to the surface of the skull, ± 3.0 lateral to the midline; (2) -0.5 posterior to bregma, -6.5 ventral to the surface of the skull, ± 2.2 lateral to the midline; (3) -1.0 posterior to bregma, -6.8 ventral to the surface of the skull, and ± 3.0 lateral to the midline; (4) -1.8 posterior to bregma, -6.4 ventral to the surface of the skull, and ± 4.0 lateral to the midline (after Dunnett, 1985). One injection ($0.6 \mu\text{g}/0.6 \mu\text{l}$ over 3 min) targeted the medial septum-diagonal band ($+0.8$ from bregma; -8.0 from surface of skull and on the midline). The injection procedure has been described previously (Mastropaulo, Nadi, Ostrowski, & Crawley, 1988). The needle was left in place 60 sec postinjection.

Other subjects ($n = 8$) received 192-SAP ($75 \text{ ng}/0.1 \mu\text{l}$) in the same eight bilateral injections into the four nucleus basalis sites, and the same single injection ($450 \text{ ng}/0.6 \mu\text{l}$) into the medial septum-diagonal band. All injections were made with a 28-gauge Hamilton syringe.

Sham control subjects ($n = 9$) had the injection needle lowered only to 3 mm into the cerebral cortex above the medial septum and nucleus basalis injection sites to avoid causing mechanical damage to the injection sites. This control group allows behavioral impairments in the two lesion groups to be compared with the performance of a group with undamaged basal forebrain neurons.

The subjects were given 12 days of recovery after surgery before behavioral testing resumed. Postsurgical survival for the IBO group was 16 out of 20. Postsurgical survival for the 192-SAP group was 7 out of 8.

Apparatus

Behavioral testing was conducted in three identical operant test chambers (MED Associates, Fairfield, VT) described in detail previously (Robinson & Crawley, 1993a, 1993b, 1994). Each test chamber had two response levers mounted on the front panel, and one on the rear panel. A white cue lamp was mounted over each response lever, and a houselight was mounted above the rear cue lamp.

Behavioral Testing

Each DNMTP trial consisted of several phases. First, a cue lamp was illuminated over one of the front levers. Following a bar-press at that lever, a delay period of 1, 5, 10, or 15 sec began. A press on the rear lever, following the completion of the delay period, turned both front cue lamps on. A press on the lever opposite from the one pressed initially (a "nonmatch to the sample") produced a water reward. A 10-sec intertrial interval (ITI) separated each of the trials. Each session consisted of 70 trials. The subjects were extensively pretrained to greater than 90% choice accuracy at the 1-sec delay, prior to surgery, as previously described (Robinson & Crawley, 1993a, 1993b, 1994).

Drugs

A threshold dose of scopolamine, $0.15 \text{ mg}/\text{kg}$ i.p., was chosen as a challenge dose for sham and lesioned rats. The scopolamine challenge was used to test for differences in cholinergic function between the two lesion methods that could be compared with deficits revealed by postmortem ChAT analyses. The dose chosen had previously been found to have no significant effect on DNMTP in normal rats (Robinson & Crawley, 1993b). Intraperitoneal injections of scopolamine or saline vehicle were given on Tuesdays and Thursdays, with intervening baseline test days on Mondays, Wednesdays, and Fridays.

Choline Acetyltransferase (ChAT) Assay

Following behavioral testing, at 18 weeks after lesioning, the subjects were decapitated under chloral hydrate anesthesia. The brains were removed, dissected as previously described (Harrington, Mobley, & Wenk, 1994), and frozen on dry ice. The anterior cortex was defined to correspond to the frontolateral sensorimo-

tor cortex, and the posterior cortical sample to correspond to the parietal-occipital cortices. These are primary projection sites for cholinergic projections originating in the nucleus basalis magnocellularis. The hippocampus is a primary projection site for cholinergic projections from the medial septum. The caudate region assessed the possibility of destruction of cholinergic interneurons important for motor control. The olfactory bulbs assayed projections from the horizontal limb of the diagonal band. ChAT activity, expressed as nanomoles of ChAT formed per hour per milligram of protein, was determined according to the method of Fonnum (1969), as has been described previously (Harrington et al., 1994). The protein content was determined by the method of Lowry, Rosenbrough, Farr, and Randall (1951).

Statistics

Statistical analyses were conducted with Version 1.0 of Superanova (ABACAS Concepts, Berkeley, CA) statistical software. Choice accuracy data were analyzed by repeated measures analysis of variance (ANOVA). Where post hoc comparisons were made between experimental groups, Dunnett's test or the Student Newman-Keuls (SNK) test was used, as will be indicated in the text. The ChAT data were analyzed with one-way ANOVAs. The post hoc tests were performed using SNK testing.

RESULTS

The subjects were matched on mean proportion correct at all delays for the last five presurgical sessions when assigned to the three surgical groups. Consequently, the three groups did not differ in performance in the final session prior to surgery [Figure 1A; $F(2,24) = 0.6$]. Four of the 20 that received IBO lesions died soon after or within the postsurgical recovery period. One of the 8 an-

imals that received 192-SAP lesions died within the postsurgical recovery period. All IBO animals required extra postsurgical attention, which consisted of injections of saline solution (1 ml every 4 h as needed for 24 h) and wet cookie mash (Nilla Wafers, Nabisco, East Hanover, NJ) or chow mash in the home cage for 1–3 days. All IBO lesioned rats also showed disrupted grooming and motor behavior 1–3 days postsurgery. By contrast, 6 of the eight 192-SAP lesioned subjects (with the exception of the one that died and the other discussed below) exhibited normal grooming, righting, and motor behavior when removed from the home cage and examined on a nearby table within 24 h postsurgery, and none required saline injections or special feedings.

Fifteen IBO group rats, seven 192-SAP group rats, and 8 sham group rats were behaviorally assessed postsurgery. Of these, 4 were discontinued over the course of DNMTTP retraining and their data were removed from the statistical analyses: one 192-SAP and 1 IBO lesioned rat completed less than 10 trials per session during the first 10 reacquisition sessions and appeared motorically impaired, and 1 IBO lesioned subject and 1 sham lesioned control became ill and died.

As shown in Table 1, the levels of ChAT were assessed in cholinergic terminal fields 18 weeks after lesioning. ChAT levels in the hippocampus of both IBO and 192-SAP groups were significantly lower than those for sham controls [$F(2,25) = 13.6, p < .0001$], with the IBO at $p < .05$ and the 192-SAP at $p < .01$ by SNK. ChAT levels for the IBO and 192-SAP groups were significantly differ-

Figure 1. Reacquisition of delayed nonmatching-to-position (DNMTTP), a working memory task, in rats lesioned with ^{192}IgG -saporin (192-SAP), ibotenic acid (IBO), or sham surgery controls (SHAM). (A) Groups did not differ in performance in the final session prior to surgery. (B) During the first block of four sessions 1–2 weeks postlesion, impairment in DNMTTP was significant when compared with sham lesioned controls in groups lesioned with the immunotoxin 192-SAP ($0.075 \mu\text{g}/0.1 \mu\text{l}$ bilaterally into the eight nucleus basalis sites, and one injection $0.45 \mu\text{g}/0.6 \mu\text{l}$ into the medial septum–diagonal band) and with the excitotoxin ibotenic acid ($0.1 \mu\text{g}/0.1 \mu\text{l}$ bilaterally into the same sites). (C) In the last block of four sessions, 15–16 weeks postlesion, no significant differences on DNMTTP performance were detected among the three groups. Note—data in Figures 1–4 are expressed as mean $\pm$ SEM. The number of animals per group for Figures 1–3 was SHAM = 8, IBO = 13, and 192-SAP = 6.

Table 1
Choline Acetyltransferase (ChAT) Levels in Cholinergic
Terminal Fields 18 Weeks Postlesioning

Brain Region	Sham Lesion	IBO Lesion	192-SAP Lesion
Hippocampus	85.7 ± 3.9	62.6 ± 3.4*	35.9 ± 12.6†‡
Anterior cortex	28.8 ± 1.1	28.1 ± 2.1	16.4 ± 3.4†
Posterior cortex	22.8 ± 3.0	25.9 ± 2.0	14.3 ± 3.4*
Caudate	74.7 ± 8.7	67.9 ± 6.6	54.5 ± 3.7
Olfactory bulbs	38.1 ± 2.8	41.3 ± 2.1	34.2 ± 6.1

Note—Data are expressed as nanomoles of ChAT formed per hour per milligram of protein ($\pm$ SEM). *Significantly different from sham lesion group ($p < .05$). †Significantly different from sham lesion group ($p < .05$). ‡Significantly different from IBO lesion group ($p < .05$).

ent from each other by SNK ($p < .05$). In the anterior cortex, ChAT was reduced in the 192-SAP group as compared with IBO or sham groups [$F(2,25) = 6.9$, $p < .004$], as indicated by an SNK post hoc test ($p < .01$). ChAT levels in the posterior cortex were also reduced in the 192-SAP group in comparison with the IBO or sham groups [$F(2,25) = 4.4$, $p < .02$], as was indicated by an SNK post hoc test ($p < .05$). ChAT levels were not significantly different among groups in the caudate nucleus [$F(2,25) = 1.4$] and olfactory bulbs [$F(2,25) = 1.2$].

As is shown in Figure 1B, at the first block of four sessions, 1–2 weeks postlesion, significant impairment in DNMTTP was seen in the IBO ($p < .0001$) and 192-SAP ($p < .0001$) groups, but not in the sham group as compared with prelesion ($p < .19$) by planned contrast [lesion $\times$ treatment interaction: $F(2,24) = 10.2$, $p < .0001$]. Additionally, when the three groups were compared on only the postsurgical first block of four sessions, the IBO and 192-SAP groups were significantly different from the sham group, and not significantly different from each other, by an SNK post hoc test ($p < .05$). At the last block of four baseline sessions (Figure 1C), 15–16 weeks postlesion, no significant difference in DNMTTP performance was detected among the three groups [$F(2,18) = 2.9$].

The time course of the behavioral deficit after the lesion is presented in Figure 2. Blocks of the means of four consecutive baseline sessions (run on Mondays, Wednesdays, and Fridays) collected over the 16-week reacquisition period indicate recovery as a function over time, depending on the delay interval. At the 1-sec delay, there was a main effect of lesion [$F(2,24) = 3.6$, $p < .05$], with the IBO group determined to be significantly different from the sham group by Dunnett's post hoc test ($p < .05$). No significant block $\times$ lesion interaction was detected [$F(2,18) = 0.8$]. At the 5-sec delay, there was a main effect of lesion [$F(2,24) = 5.3$, $p < .02$], with Dunnett's post hoc test showing that the IBO group was significantly different from sham controls ($p < .01$). At the 5-sec delay, a specific means comparison between the 192-SAP group and the sham control group approached significance ($p = .06$), and an SNK comparison indicated that the comparison between these two groups just missed statistical significance. No significant block $\times$ lesion interaction was detected [$F(2,18) = 1.3$]. At the 10-sec delay, a main effect of lesion was detected [$F(2,24) = 5.5$, $p < .02$], with Dun-

nett's post hoc test showing that the IBO group was significantly different from sham controls ($p < .01$). No significant block $\times$ lesion interaction was detected [$F(2,18) = 1.0$]. At the 15-sec delay, no significant effect of lesion [$F(2,24) = 1.6$] or significant lesion $\times$ block interaction [$F(2,18) = 0.8$] was detected.

Figure 3 shows several secondary measures of DNMTTP performance. Perseverative errors are defined as the proportion of errors that follow other errors. The sample stimulus is not randomly assigned on trials following error trials, but instead is presented over the same lever as in the previous trial. This discourages the development of position biases. Therefore, increases in perseverative errors indicate increased position bias. Trials per session measures the number of trials initiated by the subject, and is an indicator of nonspecific procedural disruption or motivational changes. The rear-lever variable-interval response rate is a retention interval distraction task but also provides a measure of general operant response rate and motoric impairment. Discrimination errors per opportunity represent presses on the lever, which is not illuminated during the sample phase. Since this is a simple light–dark discrimination that is extremely well learned, it measures reference/procedural memory. The latencies to respond to the sample and choice stimuli are recorded as simple measures of reaction time.

Both the IBO and 192-SAP groups made significantly more postlesion sample-phase discrimination errors per opportunity [$F(2,24) = 5.4$, $p < .02$] and perseverative errors [$F(2,24) = 4.6$, $p < .02$] than did the sham group (SNK $p < .05$). The IBO group showed an increased choice latency [$F(2,24) = 13.6$, $p < .0001$] when compared by SNK ($p < .05$) with both the sham and 192-SAP groups. The IBO group completed fewer trials per 60-min session [ANOVA $F(2,24) = 2.8$, $p < .08$] when assessed by IBO versus sham contrast ($p < .04$). Measures of sample response latency [$F(2,24) = 2.9$] and rear-lever retention interval response rate [$F(2,24) = 0.64$] were unaffected by the lesions.

Rats from all lesion groups were administered a sub-threshold dose of saline or scopolamine (0.15 mg/kg) in a randomized order on the 11th and 14th weeks of behavioral testing after surgery. As is shown in Figure 4, this dose was ineffective in disrupting DNMTTP in sham animals, as previously described for normal rats (Robinson & Crawley, 1993a, 1993b). Overall ANOVA showed a main effect of scopolamine [$F(1,128) = 21.9$, $p < .0001$]. No significant dose $\times$ lesion interaction was detected [$F(2,128) = 1.9$, $p < .15$]. Specific drug versus saline control contrasts for each group detected significant effects of scopolamine for the 192-SAP ($p < .007$) and IBO groups ($p < .0001$), but no significant effect for the sham group ($p < .20$).

DISCUSSION

The present results demonstrate dissociation between depletion of cholinergic markers and behavioral deficits. Profound deficits in DNMTTP choice accuracy were evi-

Figure 2. Time course for recovery of function. Shown are blocks of the means of four baseline test sessions (run on Mondays, Wednesdays, and Fridays) collected over the 16-week reacquisition period. For further explanation, see caption to Figure 1.

dent in both groups in the time soon after surgery, but diminished over the course of a 16-week recovery period. Severe depletion of ChAT in the hippocampus and cortex in the 192-SAP group and less severe depletion of ChAT in the hippocampus in the IBO group remained at 18 weeks postsurgery. Both 192-SAP and IBO treatment groups showed increased sensitivity to the disruption of choice accuracy on DNMT by a subthreshold dose of scopolamine administered during the postsurgical training period, indicating an underlying cholinergic receptor supersensitivity even when performance was returning to normal. Increased sensitivity to scopolamine on memory tasks is characteristic of Alzheimer's patients (Molchan et al., 1992).

Both the IBO and 192-SAP lesions produced delay-independent deficits in DNMT choice accuracy, primarily in the first 2 weeks of behavioral testing after lesioning, which improved gradually over the postlesion training period. The improvement of these deficits over

time has been a consistent criticism of the face validity of rodent models of Alzheimer's disease, which is a progressive disorder (for review, see Wenk & Olton, 1987). However, these delay-independent disruptions of DNMT choice accuracy are consistent with previous published reports of cholinergic lesions of the nucleus basalis (Dunnett, 1985; Dunnett, Rogers, & Jones, 1989) and treatments with muscarinic receptor antagonists (Bushnell, 1990; Dunnett, 1985; Robinson & Crawley, 1993a, 1993b; Sakurai & Wenk, 1990) on DNMT. Both IBO and 192-SAP also produced long-lasting increases in discriminative and perseverative errors. One exception is that IBO, but not 192-SAP, disrupted the rate of trial completion and choice response latency.

However, larger depletions of ChAT activity were produced by 192-SAP than by IBO. At 18 weeks after 192-SAP lesioning, the ChAT levels were significantly lower throughout the cortex (40%) and hippocampus (58%) than they were for the sham group. Other publications to

Figure 3. Secondary measures of DNMT performance. IBO and 192-SAP groups showed more postlesion sample-phase discrimination errors and perseverative errors than did the sham group. The IBO group showed an increased choice latency and completed fewer trials per 60-min session when compared with both the SHAM and the 192-SAP groups.

date have reported ChAT reductions of 76%–88% in the cortex and 64%–72% in the hippocampus at 4–5 weeks postlesion for the 192-SAP, administered separately to the medial septum or nucleus basalis (Torres et al., 1994), or up to 59%–69% reductions in the cortex at 6 weeks postlesioning by 192-SAP administered to the nucleus basalis only (Wenk, Stoehr, Quintana, Mobley, & Wiley, 1994). In the present study, we employed a longer survival time than did Torres et al. (1994) and Wenk et al. (1994), so that septohippocampal sprouting of remaining cholinergic neurons by 18 weeks may account for the smaller reductions seen in ChAT levels.

At 18 weeks postlesion, the IBO group showed ChAT levels in the cortex that were not significantly reduced in comparison with those of the sham controls, and that were significantly reduced only in the hippocampus (27%). The IBO lesion, therefore, did not produce depletion of cholin-

ergic markers to the extent that has been reported in the literature. It is also important to note that despite the reduction of ChAT activity evident at 18 weeks, both the IBO and 192-SAP groups were not substantially different from sham controls on behavioral parameters at 18 weeks.

Because some ChAT depletion was evident in the hippocampus in both IBO and 192-SAP groups, the present findings shed light on the relative contribution that the septohippocampal versus baso-cortical projections may make to successful performance of memory tasks. In the present experiment, we used combined nucleus basalis and medial septum injections of 192-SAP or IBO and, therefore, did not allow a priori comparison of the effects of separate lesions of the two structures. However, the data indicating that ChAT was depleted only in the hippocampal region in behaviorally impaired rats lesioned with IBO suggests that the septo-hippocampal cholinergic projections are

Figure 4. Scopolamine challenge. Rats from all lesion groups were administered a threshold dose of scopolamine (0.15 mg/kg i.p.). This dose was ineffective in disrupting DNMTF in sham animals, but significantly disrupted performance for the 192-SAP and IBO groups. The number of animals per group injected with saline was 6 for sham, 8 for IBO, and 7 for 192-SAP, and the number of animals per group injected with scopolamine was 7 for sham, 5 for IBO, and 5 for 192-SAP.

relatively more critical for successful completion of DNMTF. In a recent study, this hypothesis was directly tested by separately lesioning the medial septum and nucleus basalis magnocellularis with 192-SAP (670 ng/2 μ l) and assessing performance of a similar operant spatial DNMTF task in rats (Torres et al., 1994). No effects on DNMTF performance of lesions to the nucleus basalis were reported, but a mild delay-dependent disruption of DNMTF by lesions of the medial septum was observed. These findings support the interpretation that the main effects in the present experiment may be primarily attributable to septo-hippocampal cholinergic loss. Overall, the greater degree of disruption of DNMTF in the present study than reported in Torres et al. (1994) may be attributable to more extensive septal lesions in the present study (450 ng/0.6 μ l of 192-SAP).

Site injections produce mechanical damage of tissue, which can contribute to behavioral deficits. One alternative to site injections is intraventricular administration. Several studies in which i.c.v. injections of 192-SAP have been used to produce cholinergic lesions have yielded acquisition and performance deficits. Nilsson et al. (1992) reported disruption of the acquisition of a spatial discrimination in the Morris water maze, and Kelly et al. (1994) reported acquisition and performance deficits in both a place and cued version of the Morris water maze. Wiley, Berbos, Deckwith, Johnson, and Lappi (1995) found impairments in the acquisition of a step-through passive avoidance task, but not in the acquisition of conditioned freezing. Waite et al. (1995) also reported impaired water maze performance following i.c.v. administered 192-SAP. However, Waite et al. (1995) also demonstrated that the ten-fold higher i.c.v. doses required to produce cholinergic depletion in the nucleus basalis and the medial septum/nucleus of the diagonal band also produced substantial motor disturbances due to destruction of cerebellar Purkinje cells that express the p75 NGF receptor. Therefore, the profound impairments evident when 192-SAP was given

intraventricularly may be the result of motor disturbances interfering with the swimming or ambulatory behavior required for successful performance of the Morris water maze. Although the intraventricular route of administration offers the advantage of producing less mechanical tissue damage from the injector needle, interpretation of the effects is limited by the possibility that the higher doses required may also damage cerebellar neurons.

Single injections of 192-SAP into one cholinergic site produces less robust disruptions in performance of maze and passive avoidance memory tasks. Baxter et al. (1994) found that rats with 192-SAP lesions of either the medial septum or the substantia innominata, tested on a place discrimination and delayed match-to-place tasks in the Morris water maze, were not impaired in the acquisition of the spatial discrimination. Both groups were impaired in a nonspecific, delay-independent manner in the delayed match-to-place T-maze working memory task (Baxter et al., 1994). Torres et al. (1994) also found no impairment of acquisition of the Morris water maze and only slight impairment of retention of passive avoidance by 192-SAP administered separately into the medial septum/diagonal band or nucleus basalis. Wenk et al. (1994) reported that 192-SAP lesions of the nucleus basalis produced no impairments of the acquisition or performance of passive avoidance or T-maze delayed-alternation tasks. Berger-Sweeney et al. (1994) found that lesions to the septum with 192-SAP produced only mild impairments in swim-maze performance, and that performance was only impaired by doses of intraventricularly administered 192-SAP. The multiple-site injection technique, therefore, has the advantage of lower doses and better specificity to cholinergic cells of the basal forebrain, as compared with IBO or with the intraventricular route using microgram doses of 192-SAP, and more robust behavioral deficits as compared with the single-site injection approach.

In conclusion, 192-SAP is an effective tool for producing long-lasting and pronounced depletion of cortical

and hippocampal levels of cholinergic marker ChAT, and for producing substantial but transient behavioral impairments on procedural components of memory tasks. In the present study and in others, it has demonstrated usefulness in the testing of theories of cholinergic system involvement in behavior. Cholinergic drug challenges in sophisticated behavioral paradigms may help to determine the exact nature of the behavior deficits following selective cholinergic lesions made with 192-SAP.

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