Pharmacology Biochemistry & Behavior, Vol. 28, pp. 157–163. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Biochemical and Behavioral Effects of Intrahippocampal AF64A in Rats

WILLIAM D. BLAKER AND SUSAN D. GOODWIN

Virginia-Maryland Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Received 6 October 1986

BLAKER, W. D. AND S. D. GOODWIN. *Biochemical and behavioral effects of intrahippocampal AF64A in rats.* PHARMACOL BIOCHEM BEHAV **28**(2) 157–163, 1987.—AF64A (ethylcholine aziridinium, 1 nmole) injected into the dorsal hippocampus of the rat decreased choline acetyltransferase activity there by 20% without greatly affecting adjacent areas. The decrease was maximal by 3 days, and persisted for at least 3 weeks. The acetylcholine concentration at the injection site was decreased by 25–30% from 3 days to 4 weeks. Rats were trained on a continuous reinforcement (CRF) food-reinforced lever press schedule and then injected bilaterally in the dorsal and ventral hippocampus. Subsequent switching to a daily CRF-extinction schedule resulted in increased responding during extinction compared to controls which persisted for at least 13 session. However, injection after switching schedules increased it for only 2 sessions. This indicates that the persistently increased extinction responding is due mainly to impaired learned habituation to a new schedule. Most of the extinction effect of the intrahippocampal AF64A was due to its injection at the dorsal site. Separate rats which were trained on the 8-arm radial maze task (a test of short-term spatial working memory) and injected as above only showed marginally impaired task performance even at higher doses. We conclude that even relatively minor, localized, cholinergic deficits confined to the hippocampus can produce significant learning and memory impairments in situations where intermediate or long term memory formation is required.

AF64A Ethylcholine aziridinium Hippocampus Radial arm maze Extinction Acetylcholine Choline acetyltransferase

ETHYLCHOLINE mustard aziridinium ion (AF64A) has received attention as a neurotoxin with some specificity toward cholinergic neurons [15, 16, 23]. The degree of specificity appears to vary considerably, depending upon the amount, location and mode of administration, with the most specific effects being found after microinjection of suitably low doses into areas containing cholinergic nerve endings. Specifically, microinjection of AF64A into the rat hippocampus, an area known to receive a sizable cholinergic projection from the medial septum-diagonal band [5,14], produces deficits in presynaptic cholinergic markers without affecting those of noradrenergic or serotonergic neurons [16]. Since this cholinergic projection is also known to be important in memory function [2,17], we have sought to explore the effects of relatively minor, localized, AF64A-induced cholinergic deficits in the rat hippocampus on memory and learning functions. To this end, we have analyzed the regional distribution and time course of deficits in acetylcholine and choline acetyltransferase after microinjection of AF64A into the hippocampus, as well as its effects on learned habituation to extinction of a food-reinforced lever press response and on radial arm maze performance (a measure of spatial working memory).

METHOD

Animals and Surgical Procedures

Male Sprague-Dawley rats (150–200 g at the beginning of each experiment) were housed individually in quarters illuminated with 12-hr light and dark periods at a constant temperature. Food and water were freely available except in behavioral experiments (see below). For AF64A injections, rats were anesthetized with pentobarbital (45 mg/kg, IP) and stereotaxically injected at coordinates AP-3.8 mm (from bregma), L-3.3 mm, V-2.7 mm (from dura) for the dorso-medial hippocampus and at AP-5.3, L-4.8, V-5.6 for the ventral hippocampus [22]. AF64A (Research Biochemicals Inc., Wayland, MA) was prepared the day of the injections [9] and was administered in a volume of 1 μl over a period of one minute at each injection site.

Behavior

In experiments measuring habituation to extinction, rats were food deprived for 48 hours and then trained on a continuous reinforcement schedule (CRF, 15 min per day) to lever press for food pellets (45 mg; Lab Animal Food, P.J. Noyes Co., Lancaster, PA). The training was performed using 21.5×21.5×28 cm chambers with Plexiglas sides and lid, a 5 cm wide response lever 7.6 cm above the grid floor and a food pellet cup 5 cm above the grid flood (Lafayette Instrument Co., Lafayette, IN). Chambers were housed in illuminated, sound-attenuated boxes and the reinforcement schedules and recording of responses were implemented by computer interface. The diet of the rats was supplemented with additional food (15–17 g daily) to maintain a rate of weight gain of 3 to 5 g/day throughout the experiment. The rats were trained on the CRF schedule for 6–7 sessions. The

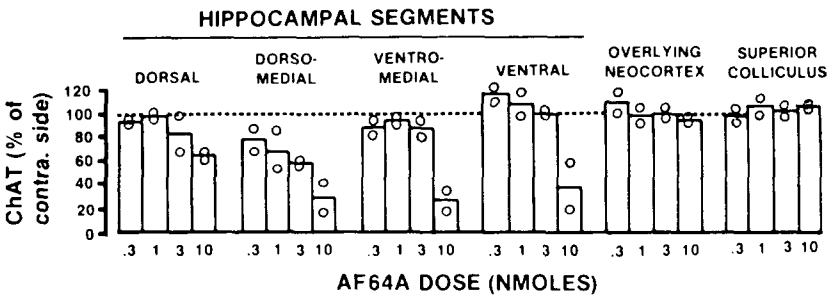

FIG. 1. Effect of unilateral AF64A injection into the dorso-medial hippocampus on choline acetyltransferase activity. Two rats per dose were killed one week post-injection.

TABLE 1

EFFECT OF INTRAHIPPOCAMPAL AF64A ON REGIONAL LEVELS OF CHOLINE ACETYLTRANSFERASE ACTIVITY AT 1 WEEK POST-INJECTION

Tissue	ChAT Activity (mean percent) of contralateral side ± SEM) (N)	p of Paired t-Test
Dorsal hippocampus	96 ± 5.5 (7)	—
Dorso-medial hippocampus	81 ± 5.9 (7)	0.023
Ventro-medial hippocampus	91 ± 3.3 (7)	0.029
Ventral hippocampus	98 ± 3.7 (7)	—
Neocortex	97 ± 2.9 (6)	—
Superior colliculus	105 ± 1.2 (7)	0.005

TABLE 2

TIME COURSE OF EFFECT OF INTRAHIPPOCAMPAL AF64A ON ChAT ACTIVITY AT THE DORSO-MEDIAL INJECTION SITE

Time Post-Injection	ChAT Activity (mean percent of contralateral side ± SEM) (N)	p of Paired t-Test
3 days	80 ± 4.8 (5)	0.011
1 week	81 ± 5.9 (7)	0.023
2 weeks	89 ± 2.9 (5)	0.016
4 weeks	92 ± 3.3 (5)	—

schedule was then shifted to a continuous reinforcement-extinction (CRF-EXT) schedule in which 5 minutes of continuous reinforcement were followed by 10 minutes during which no reinforcements were given. This schedule was maintained for the remainder of each experiment. In one experiment, various doses of AF64A were injected bilaterally into the dorso-medial and ventral hippocampus before initiation of the CRF-EXT schedule, while in another experiment the injection was made after 6 CRF-EXT sessions had been administered. In both cases, 3–4 days of post-surgical recovery was allowed before recommencing the behavioral training. Percentage of time responding was calculated by subtracting the time during which no responding occurred ("silent" periods of at least 30 sec) from the total time of the session and dividing this difference by the length of the session. Significant differences between dosed and control groups when examining each test day independently were determined either by Student's t-test (one dosed group) or by Dunnett's multiple comparison test (two dosed groups) [25]. Such an analysis allows the determination of the temporal pattern of significant differences over time (e.g., is the effect transient). Significant differences between dosed and control groups when examined over the entire course of the post-injection period were determined by linear contrasts using a general linear models procedure [18]. This analysis is more sensitive, allowing for fewer animals per group, and analyzes only for overall differences over the entire time period studied.

In experiments measuring radial maze performance, rats were food-deprived for 48 hours and then placed in the eight-arm radial maze [20]. Briefly, the maze consisted of a central, octagonal, 28 cm diameter platform from which eight 10×80 cm runaway arms extended like spokes of a wheel. Each arm was surrounded by an opaque wall and contained a depressed food cup 3 cm from the end. The entire apparatus was elevated 36 cm off the floor in a well-lit 2×3 m room containing permanent visual cues (doors, cabinets, etc.) and a white noise sound masking source. Each food cup contained a food pellet (190 mg; Lab Animal Food, P.J. Noyes Co., Lancaster, PA) and the diet of the rats was supplemented as described above. After about 15 training sessions of a maximum of 10 minutes each, those rats which consistently made less than seven correct choices in the first eight arms entered, or those which consistently made more than half their choices by entering adjacent arms were eliminated from the experiment. The rats were then injected with AF64A as described above and after two days of recovery, the training recommenced. Significant differences between dosed and control groups for each test day were determined either by the Wilcoxon rank sum test (one dosed group) or Dunn's nonparametric comparison of several groups to a control (two dosed groups) [7].

Biochemistry

At 3 days to 4 weeks after unilateral injection of AF64A into the dorso-medial hippocampus, choline acetyltransferase (ChAT) and acetylcholine (ACh) levels were determined in four equal ventral-to-dorsal hippocampal segments, the superior colliculus and the ca. 10 mg segment of neocortex through which the injection needle passed. The hippocampal

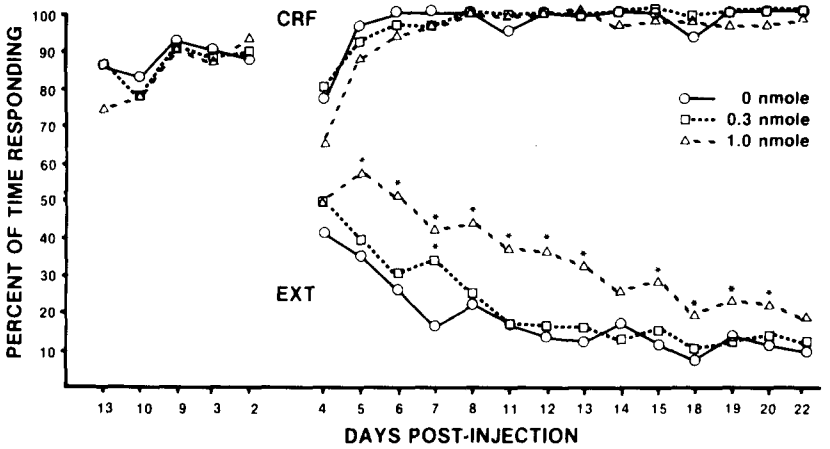

FIG. 2. Effect of intrahippocampal AF64A on responding during continuous reinforcement (CRF) and extinction (EXT). Values are the mean of nine rats per group. *Significant difference from 0 nmole group ($p<0.05$).

TABLE 3

EFFECT OF INTRAHIPPOCAMPAL AF64A ON ACETYLCHOLINE LEVELS AT THE DORSO-MEDIAL INJECTION SITE AT VARIOUS TIMES POST-INJECTION

Time Post-Injection	ACh Concentration (mean percent of contralateral side ± SEM) (N)	p of Paired t-Test
3 days	73 ± 10 (6)	0.039
2 weeks	69 ± 8 (5)	0.025
4 weeks	74 ± 7 (6)	0.013

dissection of each segment included the dentate, areas CA1 to CA3, and subiculum, but not the pre-subiculum. For ChAT assays, the rats were killed by decapitation, the brains quickly removed, and brain areas dissected on ice and stored frozen. The specific activity of ChAT in whole tissue homogenates (nmoles product/mg protein/hour) was determined radiochemically [6]. Protein was assayed by the method of Lowry *et al.* [12]. Significant differences were determined by two-sided paired t-test, comparing injected side vs. contralateral side values for each rat. In the behavioral experiments utilizing bilateral injections, values from control and injected rats were compared for selected brain areas.

For ACh assays, rats were killed by head-focussed microwave irradiation (6 kW Vivostat, Cober Electronics, Stamford, CT) and the dissected brain areas stored frozen. The ACh concentrations (nmoles/mg protein) of the whole tissues (mean of triplicate assays) were determined by radioreceptor assay [3]. Significant differences were determined as described above.

RESULTS

Biochemistry

Figure 1 shows that at low doses (0.3 to 3 nmoles), AF64A decreased the ChAT activity in the injection area by one week post injection without greatly affecting other hippocampal areas. The neocortex and superior colliculus were unaffected, even at high doses. The 10 nmole dose led to pronounced ChAT deficits at the injection site as well as at more ventral levels, most likely due to non-specific damage to fibers passing through the injection area. None of the doses led to mortality when injected unilaterally at the dorso-medial site, but even 3 nmoles produced significant mortality when injected bilaterally at dorsal and ventral hippocampal sites (see below).

The 1 nmole dose of AF64A was chosen for more detailed biochemical study since this dose produces behavioral deficits in the extinction paradigm (see below). A larger group of rats was injected in the dorso-medial hippocampus and the distribution of ChAT deficits was determined 1 week post-injection. The results shown in Table 1 confirm the previous findings, in that the deficits were largely confined to the injection area. The statistically significant increase in ChAT in the superior colliculus is due to the unusually small standard error and is probably not biologically significant. A time course of the ChAT activity at the injection site was performed and the results are shown in Table 2. There was a rapid ($\leq$3 days) decrease in ChAT. Although there appeared to be a trend toward a gradual recovery of activity levels over the pursuing weeks, the magnitudes of the deficits were not significantly different from one another by one-way ANOVA.

In separate rats, the time course of ACh level deficits was determined at the dorso-medial hippocampal injection site and the results are shown in Table 3. A 25–30% decrease in ACh levels was apparent at three days post-injection and persisted through four weeks.

Extinction Responding

Rats were trained on a 15-minute continuous reinforcement (CRF) food-reinforced lever press paradigm. After stabilization of the performance, the rats were injected with either 0, .3 or 1.0 nmole AF64A per injection site bilaterally into the dorso-medial and ventral hippocampus. The rats were then switched to a 5 minute continuous reinforcement 10 minute extinction (CRF-EXT) paradigm and response parameters in both phases of the paradigm were measured over

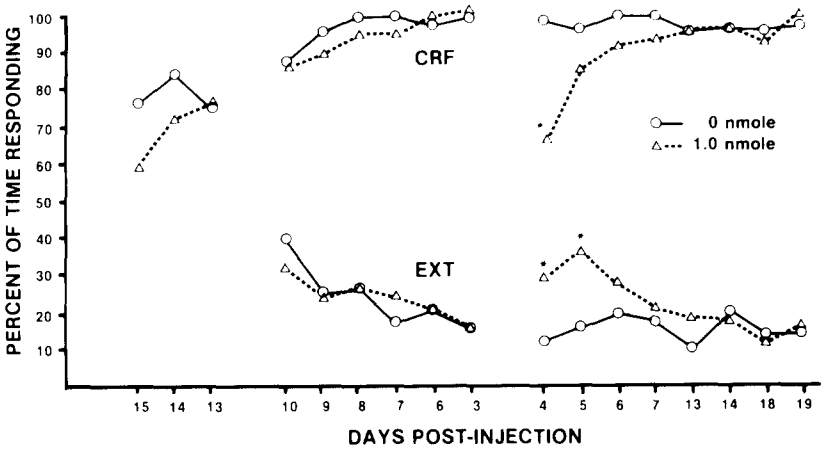

FIG. 3. Effect of intrahippocampal AF64A on responding during continuous reinforcement and extinction (extinction schedule initiated before toxin treatment). Values are the mean of nine rats per group. *Significant difference from 0 nmole group ($p<0.05$).

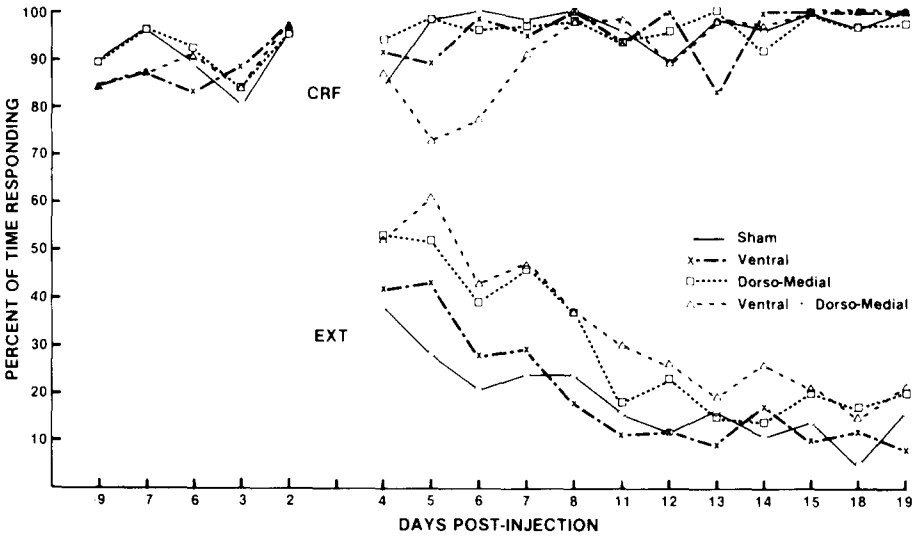

FIG. 4. Regional effect of intrahippocampal AF64A on responding during continuous reinforcement and extinction. Values are the mean of 5–6 rats per group.

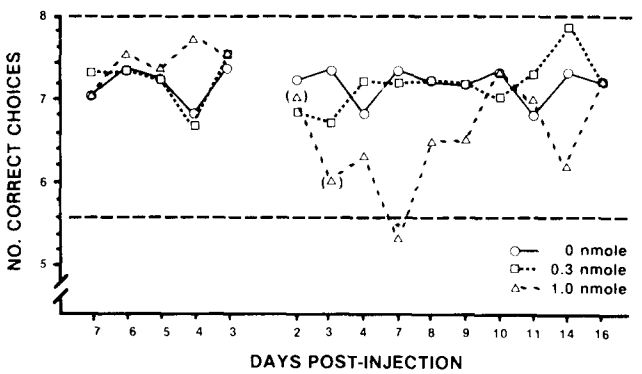

FIG. 5. Effect of intrahippocampal AF64A on radial maze performance. Values are the mean of six rats per group (values in parentheses are from less than six rats due to erratic post-injection behavior). Line at 5.6 indicates level of random arm choice. *Significant difference from 0 nmole group ($p<0.05$).

subsequent sessions. The results are shown in Fig. 2. While the percent of the time spent responding in the CRF phase was not different among the three groups, this parameter was increased during the EXT phase in the 1.0 group, but not in the 0.3 group. This resistance to extinction persisted through at least three weeks of testing (ca. 13 sessions). Almost identical results were obtained when response rates were used as the response parameter, i.e. the 1.0, but not the 0.3, group showed an increase in responding only in the EXT phase which persisted for ca. 3 weeks (data not shown).

Since this persistent increase in extinction responding could be due to increased response perseveration or impaired learned adjustment to the post-injection extinction paradigm, a second experiment was performed to distinguish between these two possibilities. Rats were trained on a 15 minute CRF paradigm, switched to the CRF-EXT paradigm, trained to stabilization, and then injected with 1 nmole AF64A as in the previous experiment. The CRF-EXT paradigm was continued and the results are shown in Fig. 3.

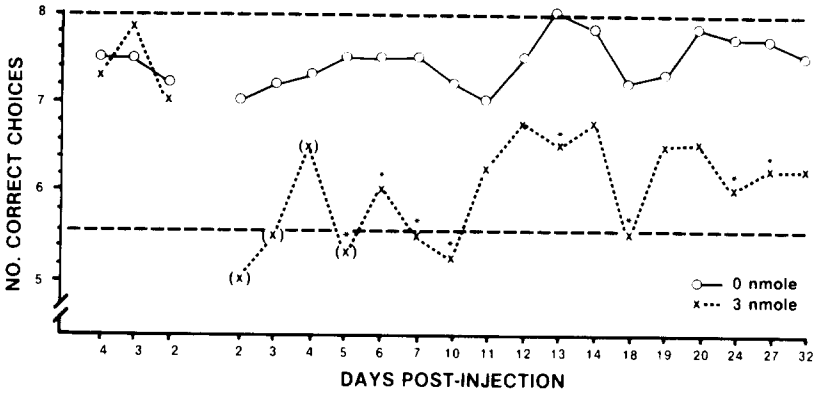

FIG. 6. Effect of intrahippocampal AF64A on radial maze performance. Values are the mean of six rats in the 0 nmole group and four in the 3 nmole group (values in parentheses are from less than four rats due to erratic post-injection behavior). *Significant difference from 0 nmole group ($p<0.05$).

Although EXT responding was increased post-injection, it persisted for only 2 sessions the first of which also showed CRF responding deficits. Again, response rates showed the same results (data not shown). The rats thus clearly have difficulty in habituating to extinction if initiated after the injection, but have little difficulty in retaining the habituation if established before the injection. We interpret these results as indicating that the persistently increased extinction responding found when switching paradigms after AF64A injection is due mainly to impaired learned adjustment to the new paradigm. To test for proper AF64A administration in these rats, ChAT levels at the dorso-medial injection sites were assayed 4 weeks post-injection. Since the injections were bilateral, the comparison was between injected and control rats and the injected values were found to be significantly decreased to 84±7% of control. The trend toward a greater deficit found here than in the time course experiment (Table 2) may be due to a small contribution from the accompanying ventral hippocampal injection.

The regional specificity of the extinction effect was tested and the results are shown in Fig. 4. The groups injected in the dorso-medial hippocampus and in both the dorso-medial and ventral hippocampus showed increased extinction responding compared to the control group over the course of the post-injection analysis period ($p=0.007$ and $p=0.0008$, respectively). The group injected at only the ventral hippocampus was not significantly different from the sham control group ($p=0.66$). Proper AF64A administration at the ventral sites was shown by the fact that the ventrally injected and the ventrally plus dorso-medially injected rats showed significantly decreased ventral hippocampal ChAT levels at 3 weeks post injection (69±17% and 67±14% of control, respectively). Dorsally injected rats did not show significant effects at the ventral hippocampus. Thus most, if not all, of the effect of intrahippocampal AF64A on extinction responding is due to injection at the dorso-medial site.

Radial Maze Performance

Whereas the extinction experiments showed a decrement in learning requiring a memory span over a period of several days, the next experiments with radial maze performance were carried out to test for impairment of spatial working memory which spans only a few minutes.

Rats were trained on an 8-arm radial maze to stabilization and then injected with 0, 0.3 or 1.0 nmole AF64A bilaterally into the dorso-medial and ventral hippocampus. Radial maze performance was assayed over subsequent sessions and the results are shown in Fig. 5. The 0.3 group showed no impairment in maze performance, while the 1.0 group showed some impairment which nevertheless was uneven and did not consistently reach statistical significance. Subsequently, a second similar experiment was performed with a dose of 3.0 nmole AF64A. This dose did produce a persistent deficit in maze performance which more consistently reached statistical significance (Fig. 6). However, this dose must be close to the LD_{50} since 3 of the 7 injected rats died 1–2 days post-injection. Clearly, the effects of hippocampal AF64A are less pronounced on radial maze short term spatial memory than on the more long term habituation to extinction. Both at the 1.0 and 3.0 doses, decrements in radial maze performance was often seen after a lapse in testing of 2–3 days (Figs. 5–6). In an attempt to exploit this, an experiment was performed in which the post-injection testing (after a 1.0 nmole dose) was administered only on days 2,7,10,14,17, and 21 (data not shown). However, the performance of the dosed rats was still not consistently different from controls and did not reach random choice levels (7 controls, 6 dosed). Control means ranged from 6.8 to 7.6 while dosed means ranged from 6.0 to 6.7. Only on two of the six testing days was the performance of the dosed rats significantly worse than the controls by the Wilcoxon Test.

DISCUSSION

Literature results indicate that the specificity of the cholinotoxic effect of AF64A is dependent on the amount, mode and location of the intracranial injection. It appears that cholinergic nerve terminals are much more sensitive to AF64A toxicity than are cholinergic cell bodies and that the specificity of the toxin is most readily achieved by suitable doses in microinjections confined to cholinergic terminal areas. For example, injection of 0.1–0.5 nmoles into the striatum, which is rich in cholinergic nerve endings, produces minimal nonspecific damage and moderate decreases in cholinergic indices, while the same dose range produces unacceptable nonselective damage when injected into the nucleus basalis [13]. Others have similarly reported

destruction of cholinergic perikarya after local injection into the nucleus basalis and medial septum only at dose levels which also produce nonspecific damage [1, 10, 19]. In contrast, injections into the striatum and hippocampus have consistently shown dose-response curves having regions of good specificity for cholinergic indices [16,23]. Injection of 8 nmoles into the striatum, which decreases presynaptic cholinergic, but not GABAergic or dopaminergic markers, leads to only a narrow (0.1 mm) zone of gliosis and no loss of cholinergic cell bodies as defined as cells staining intensely for acetylcholinesterase shortly after DFP treatment [23]. In addition, intracerebroventricular injections are likely to lead to areas of nonspecific damage at the doses needed to reduce cholinergic markers in regions not immediately bordering the ventricular surfaces. Indeed, such damage close to the intraventricular injection site has been reported [9]. Nonspecific damage found when applying an arbitrary dose of the compound to areas largely devoid of cholinergic neurons or nerve endings [11] is not relevant to the determination of the specificity of AF64A since dose levels which would be selective for cholinergic components in the injected area cannot be determined.

The decreases in ChAT and ACh found in the present study, although significant, are more modest than those found by others using similar doses of the toxin [16,26]. This discrepancy may be due to systematic differences in toxin preparation and purity. Nevertheless, these cholinergic deficits confined to the hippocampus are sufficient to produce behavioral effects which are consistent with those found by others with intrahippocampal [26] and intracerebroventricular AF64A as well as with other methods which disrupt the septal-hippocampal cholinergic system. Thus, intracerebroventricular AF64A produces radial arm maze performance deficits [27] as do bilateral, but not unilateral, lesions of the fimbria-fornix [21], bilateral dorsal hippocampal lesions [28], and the systemic administration of the muscarinic antagonist scopolamine [24]. However, we have found that habituation to extinction is more consistently affected than is the short term memory component of the radial arm maze. The prolonged increase in extinction responding when AF64A was injected prior to exposure to the extinction paradigm is similar to findings with hippocampal [8] or septal [4] lesions and is most likely due to learning impairments.

The advantage of the present intrahippocampal AF64A microinjection approach to the study of hippocampal cholinergic contributions to memory and response processes is that behavioral effects can be produced by relatively modest, localized hippocampal cholinergic deficits. Such deficits cannot be attained by more nonspecific lesion methods (e.g., kainic acid or tract cutting) which affect neurotransmitter systems indiscriminately, by localized microinjections of anticholinergics which are not long lasting, nor by chronic systemic anticholinergic administration which produces non-localized effects.

ACKNOWLEDGEMENTS

The authors wish to thank Bronwen Nishikawa for her assistance in the regional specificity behavior study. This work was supported by grants to W.D.B. from the Pharmaceutical Manufacturer's Association Foundation and the Alzheimer's Disease and Related Disorders Association and by a VA-MD Regional College of Veterinary Medicine Student Summer Research Fellowship to S.D.G.

REFERENCES

1. Asant, J. W., A. J. Cross, J. F. W. Deakin, J. A. Johnson and H. R. Stater. Evaluation of ethylcholine mustard aziridinium ion (ECMA) as a specific neurotoxin of brain cholinergic neurones. *Br J Pharmacol* **80:** 573, 1983.
2. Brito, G. N. O., B. J. Davis, L. C. Stoop and M. E. Stanton. Memory and the septo-hippocampal cholinergic system in the rat. *Psychopharmacology (Berlin)* **81:** 315–320, 1983.
3. Ehlert, F. J., W. R. Roeske and H. I. Yamamura. A simple and rapid radioreceptor assay for the estimation of acetylcholine. *Life Sci* **31:** 347–354, 1982.
4. Ellen, P., G. Gillenwater and W. K. Richardson. Extinction responding by septal and normal rats following acquisition under four schedules of reinforcement. *Physiol Behav* **18:** 609–615, 1977.
5. Fibiger, H. C. The organization and some projections of cholinergic neurons of the mammalian forebrain. *Brain Res Rev* **4:** 327–388, 1982.
6. Fonnum, F. A rapid radiochemical method for the determination of choline acetyltransferase. *J Neurochem* **24:** 407–409, 1975.
7. Hollander, M. and D. A. Wolfe. *Nonparametric Statistical Methods*. New York: John Wiley and Sons, 1973.
8. Jarrard, L. E., R. L. Isaacson and W. O. Wickergren. Effects of hippocampal ablation and intertrial interval on runway acquisition and extinction. *J Comp Physiol Psychol* **57:** 442–444, 1964.
9. Jarrard, L. E., G. J. Kant, J. L. Meyerhoff and A. Levy. Behavioral and neurochemical effects of intraventricular AF64A administration in rats. *Pharmacol Biochem Behav* **21:** 273–280, 1984.
10. Johnson, D. A., B. E. Polenchar, A. L. Beggs, P. R. Sandberg and M. M. Patterson. Avoidance behavior following electrolytic, AF64A and kainic acid lesions of the septum. *Soc Neurosci Abstr* **11:** 636, 1985.
11. Levy, A., G. J. Kant, J. L. Meyerhoff and L. E. Jarrard. Noncholinergic neurotoxic effects of AF64A in the substantia nigra. *Brain Res* **305:** 169–172, 1984.
12. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
13. McGurk, S. R. and L. L. Butcher. Cholinergic neuropathology following intracerebral infusion of ethylcholine mustard aziridinium (AF64A). *Soc Neurosci Abstr* **11:** 1240, 1985.
14. McKinney, M., J. T. Coyle and J. C. Hedrum. Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. *J Comp Neurol* **217:** 103–121, 1983.
15. Mantione, C. R., A. Fisher and I. Hanin. The AF64A-treated mouse: possible model for central cholinergic hypofunction. *Science* **213:** 579–580, 1981.
16. Mantione, C. R., M. J. Zigmond, A. Fisher and I. Hanin. Selective presynaptic cholinergic neurotoxicity following intrahippocampal AF64A injection in rats. *J Neurochem* **41:** 251–255, 1983.
17. Mitchell, S. J., J. N. P. Rawlins, O. Steward and D. S. Olton. Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats. *J Neurosci* **2:** 292–302, 1982.
18. Morrison, D. F. *Multivariate Statistical Methods*. New York: McGraw-Hill, 1976, pp. 205–216.
19. Myles, L. A., M. Steingart, R. J. Rylett and E. H. Colhoun. Effect of injection of choline mustard into medial septal area of rat brain on biochemical and behavioral parameters. *Soc Neurosci Abstr* **10:** 1069, 1984.
20. Olton, D. S. and R. J. Samuelson. Remembrance of places passed: Spatial memory in rats. *J Exp Psychol [Anim Behav Proc]* **2:** 97–116, 1976.

21. Olton, D. S., J. A. Walker and W. A. Wolf. A disconnection analysis of hippocampal function. *Brain Res* **233:** 241–253, 1982.
22. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
23. Sandberg, K., I. Hanin, A. Fisher and J. T. Coyle. Selective cholinergic neurotoxin: AF64A's effects in rat striatum. *Brain Res* **293:** 49–55, 1984.
24. Stevens, R. Scopolamine impairs spatial maze performance in rats. *Physiol Behav* **27:** 385–386, 1981.
25. Tallarida, R. J. and R. B. Murray. *Manual of Pharmacological Calculations with Computer Programs*. New York: Springer-Verlag, 1981.
26. Walsh, T. J., D. L. Dehaven, H. A. Tilson, R. B. Mailman, A. Fisher and I. Hanin. Cholinergic lesions of the hippocampus produce long-term alterations of reactivity and cognitive function. *Soc Neurosci Abstr* **10:** 257, 1984.
27. Walsh, T. J., H. A. Tilson, D. L. DeHaven, R. B. Mailman, A. Fisher and I. Hanin. AF64A, a cholinergic neurotoxin, selectively depletes acetylcholine in cortex and hippocampus, and produces long-term passive avoidance and radial-arm maze deficits in the rat. *Brain Res* **321:** 91–102, 1984.
28. Winocur, G. Radial-arm-maze behavior by rats with dorsal hippocampal lesions: effects of cuing. *J Comp Physiol Psychol* **96:** 155–169, 1982.