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Behavioral and Neurochemical Effects of Intraventricular AF64A Administration in Rats[1,2]

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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(2)273–280, 1984.—Ethylcholine aziridinium ion solution (AF64A), a putative specific cholinergic neurotoxin, was injected bilaterally into the lateral ventricles of rats. Following administration of 3 or 6 nmoles of AF64A, drinking and eating were depressed but returned to normal levels after several days; increased activity in the 6 nmole group persisted throughout the 21 days of observations. Performance on complex place and cue tasks indicated that injected animals were impaired in reference memory only on the place task, but working memory was impaired on both tasks. Neurochemical measurements in a separate group of animals one week after AF64A injections found large depletions of acetylcholine in hippocampus and corpus striatum, but not depletions of norepinephrine (hippocampus) or dopamine (striatum). Histological examination of the injection site revealed extensive damage to the fimbria-fornix similar to that seen after electrolytic lesions. Since the behavoral and neurochemical changes are similar to those previously found following fimbria-fornix lesions, it is concluded that the present results are possibly due to non-specific lesion effects of the neurotoxin rather than a specific effect on cholinergic systems.

AF64A Ethylcholine aziridinium ion Radial arm maze Memory deficits Fimbria-fornix lesion Acetylcholine depletion

RECENTLY, a number of choline analogues have been suggested as potential tools in developing selective animal models of central cholinergic hypofunction [6]. Choline mustard aziridinium ions were found to irreversibly inhibit the high-affinity transport of choline in rat forebrain synaptosomes [21]. At the same time, one of the drugs, ethylcholine aziridinium ion (AF64A), was found to have similar effects *in vivo* when injected intracerebroventricularly (ICV) in mice [7]. This compound has recently been used in several studies, both *in vitro* and *in vivo* [1, 22, 27].

Because of its persistent cholinergic effects, AF64A has also been suggested as a promising drug to use in generating a model for Alzheimer's disease [8]. Senile dementia of Alzheimer's type (SDAT) is characterized behaviorally by a general decline in cognitive functions. In the present study, we set out to correlate the neurochemical effects of ICV administration of AF64A, some of which have already been documented, with its behavioral effects.

In preliminary experiments, the LD_{50} of AF64A, based on the lethality during the first week following ICV injection in rats, was determined to be 15 nmoles (7.5 nmoles in each ventricle). Therefore, for the behavioral and neurochemical experiments which followed, a much lower dose of the compound was used.

In the behavioral experiments, activity, eating and drinking were measured continuously for 24-hour periods in the home cage, while learning and memory were studied using a radial arm maze together with a procedure that permits determining two kinds of learning (place and cue) and two memory functions (working memory and reference memory) [11].

In the place task, the rat learns to choose four out of eight similar arms where all arms remain in the same spatial location and the same arms are baited from trial to trial. In the cue task, textured floor inserts in the eight arms are moved in a random order from trial to trial and the rat is rewarded for

[1]The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

[2]In conducting the research described in this report, the investigator(s) adhere to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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choosing the same four cues. Working memory (WM) involves flexible associations that change from trial to trial, whereas reference memory (RM) involves remembering information that is applicable across trials (see [10]). In our procedures, WM involves remembering which baited arms of the maze (or cues) have been visited within a trial while RM refers to choices of baited and unbaited arms or cues.

Neurochemical studies of AF64A-induced changes in the levels of acetylcholine (ACh), norepinephrine (NE) and dopamine (DA) in various brain areas were carried out in order to correlate them with any behavioral changes observed, as well as to establish that, in our hands, the neurochemical effects of the drug were similar to those previously reported [7].

Histological analysis of the brains of the injected rats concluded these experiments.

METHOD

Animals and Surgical Procedures

Sprague-Dawley male rats (250–300 g) were housed individually in a temperature and light controlled room. Food and water were freely available, except for the rats trained in the radial maze which were food restricted as previously described [11]. For the surgical procedures, animals were anesthesized with pentobarbital (45 mg/kg IP). Following a midline incision, holes were drilled in the skull overlying the lateral ventricles. Stereotaxic procedures were used with a Hamilton syringe to make the injections at the following coordinates: AP=−1.0; ML=±1.6; DV=−4.3 [18]. In Experiments 1 and 2, injections of 0.5 μl of either AF64A solution (3 or 6 mM) or the vehicle were made into each ventricle over a 3 min period and the needle was left *in situ* for two additional min to prevent spread up the tract. Thus animals in the high dose group received a total of 6 nmoles of AF64A and rats in the low dose group received 3 nmoles. In Experiment 3, in order to check the effect of the dose and the volume, 3 nmoles and 1 nmole of AF64A were injected bilaterally in a total volume of 1 or 3 μl.

AF64A Preparation

AF64A was freshly prepared from acetyl AF64 as previously described [7]. The purity of the starting material was verified by NMR analysis. Acetyl AF64 (stored in a desiccator under vacuum) was rapidly dissolved in distilled water (1.5 mg/ml). The pH was then carefully adjusted to 11.5 (never to exceed 11.7) with a small amount of 10N NaOH (approximately 1 μl/ml) and maintained at 11.5 for 25 min by addition of 1 N NaOH as needed (approximately 10 μl/ml). Then, the pH was reduced to between 5 and 7.4 with 6 N HCl (approximately 1.5 μl/ml) and finally adjusted to pH 7.4 with solid $NaHCO_3$. Distilled H_2O was added to bring the final concentration of AF64A to 6 mM (representing 1.38 mg/ml of the original material). Lower concentrations were prepared by dilutions of this stock solution with distilled H_2O. A similar procedure was followed to prepare the vehicle for control injections. Drug solutions were kept on ice until used, usually within 6 hours.

Experimental Design

Three experiments using different groups of rats were performed. In Experiment 1, 24 hr activity and food and water consumption were determined. In Experiment 2, radial arm maze performance of AF64A-treated rats was tested. In Experiment 3, changes in the levels of three neurotransmitters (ACh, NE, DA), following four dose-volume combinations of AF64A injections, were determined. Histological examinations were made on the rats used in Experiments 1 and 2 and on a parallel group in Experiment 3.

Experiment 1: Activity, Eating and Drinking

Activity, eating and drinking were determined using the Activity, Eating and Drinking (AED) System [12]. The AED consists of 18 individual cages equipped with sensors that permit measurement of movement, when the animals eat and drink, and the amount of food and water consumed. The sensors are sampled every second, information is stored in an Ohio Scientific Microprocessor, and totals for each parameter are printed each hour. For purposes of the present study, the 12 hr night (1800–0600) and 12 hr day (0600–1800) totals were calculated. In addition, the total amount of food and water consumed by each animal per 24 hr was totaled.

In Experiment 1, 18 rats were habituated to the AED for 10 days. Following this baseline period, animals were divided into three groups such that the baseline data for each group was similar. The high dose group was injected with 6 nmoles of AF64A (n=7), the low dose group was injected with 3 nmoles (n=6) and the third group was injected with vehicle (n=5). All animals were immediately returned to the AED cages. Measurements were continued for 21 days, after which the animals were perfused and their brains saved for histology.

Experiment 2: Radial Maze

The radial arm maze used in this study had a hexagonal center platform with eight arms radiating from the center [11]. In the place task, the eight similar arms remained in the same location with respect to extramaze cues, and the same four arms were consistently baited for any one animal. In the intramaze cue task, seven removable inserts of different materials (aluminum, cloth, ceiling tile, chicken wire, screen, carpeting, and sandpaper) were moved in a random order from trial to trial. Any one animal had the same four inserts (cues) baited from trial to trial.

The procedures for deprivation and preliminary training were similar to those previously described [11]. Preoperative testing included two daily trials on each task (place and cue), with the testing order being alternated over days. A trial consisted of baiting each of the four correct arms (or cues) with three drops of a 23% sucrose solution and placing the rat in the center of the platform. The animals remained on the maze until all four reinforcements had been received or until 16 choices had been made or until 5 min had elapsed, whichever occurred first. Choices of arms were recorded. Training trials were continued until the animals reached a criterion of four errorless trials out of five on each task. Of the 16 animals that completed training, six were assigned to the high dose group (6 nmoles), six to the low dose group (3 nmoles) and four served as vehicle controls. The operations were carried out following the procedures described above.

After the operations, the rats were fed ad lib for three days and then placed back on the deprivation schedule. Testing on both the place and cue tasks was resumed after one week using the same procedures as described above. Testing of retention was continued for six weeks with each rat receiving 10 trials per week on each task.

After 60 postoperative trials where the animals were rewarded for choosing the same arms and cues as those rein-

forced in the original training before the operations, reversal learning was carried out. This involved baiting the opposite four arms and four cues. Reversal training was continued for three weeks by which time the animals had received 30 reversal trials on each task.

After final testing, all operated animals were sacrificed and the brains saved for histology.

Experiment 3: Neurochemistry

Preliminary histological examination of the rats used in Experiments 1 and 2 revealed extensive damage at the injection site. Therefore, in order to determine optimal conditions for AF64A injection, several dose-volume combinations, including the one used in Experiments 1 and 2, were used.

Thirty rats were used in this experiment. Six rats in each group were injected bilaterally into the ventricles with either 0.5 or 1.5 μl of AF64A solution (0.3, 1.0, or 3.0 mM) or 1.5 μl of the vehicle on each side. Thus, by various combinations we had two groups that received 1 nmole of AF64A (either in 1 μl or 3 μl) and two groups that were injected with 3 nmoles of AF64A (either in 1 μl or 3 μl).

One week following surgery, rats were sacrificed by high power microwave irradiation in order to prevent postmortem degradation of acetylcholine [24,25]. The microwave system used a modified Varian PPS-2.5 power generator with an output of 2.5 Kw at a frequency of 2450 MHz [14,16]. Following microwave irradiation for 5 sec, the rats were decapitated. Brain regions were dissected, weighed, and sonicated in 1.0 to 2.5 ml of 50 mM Tris-Hepes buffer (pH 7.4). The homogenates were centrifuged at 12,000 g at 4°C and the supernatants were acidified to pH 4 with 1.0 N perchloric acid and then stored at −20°C until assayed for acetylcholine, dopamine, and norepinephrine.

Acetylcholine was measured using a radioreceptor assay [4]. Briefly, samples (vigorously vortexed after thawing), or standards were incubated for 90 min at 0°C with a radioactive muscarinic agonist (^{3}H-cis-methyldioxolane, New England Nuclear, 38.1 Ci/mmole) and acetylcholine receptors prepared from pooled rat cortex tissue. Non-specific binding was determined by incubating some tubes in the presence of atropine (10^{-6} M).

Dopamine and norepinephrine were determined by radioenzymatic assay as previously described [3]. Twenty-five μl of sample were incubated with ^{3}H-S-adenosylmethione (New England Nuclear 15 Ci/mmole) and catechol-o-methyl transferase prepared in our laboratory. Methylated catecholamines were separated from excess ^{3}H-S-adenosylmethione by solvent partitioning. Periodate oxidation separated labelled norepinephrine and dopamine. Internal standards were used for each brain region.

Histology

After final testing, all operated animals used for behavioral testing and some additional injected animals were perfused with physiological saline solution followed by 10% formalin. The brains were removed, soaked in a neutralized formalin solution for several days and then embedded in egg yolk. The brains were sectioned in the coronal plane at 30 μm, and every tenth section was stained with a cresyl violet stain for cell bodies. In addition, the Fink-Heimer silver stain was used on selected sections in order to identify degenerating axons [5].

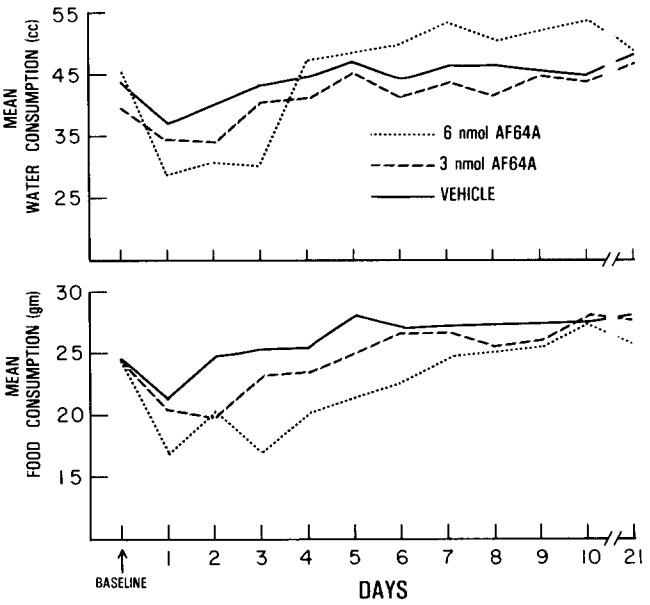

FIG. 1. Mean pre- and postoperative consumption of water (top) and food (bottom) for the groups.

RESULTS AND DISCUSSION

Experiment 1

Several animals (two from the 6 nmoles group and one from the 3 nmoles group) died within four days after drug injection. While it is not known why the animals died, most AF64A-injected animals were observed to have seizures on recovering from the anesthesia and the animals were adipsic and aphagic (see below). Of the three animals that did not survive, one died within one day while the other two died three and four days after the injection. Because of the loss of these animals, analysis of behavior in the AED system was based on data from five animals from each group.

The effects of ICV injection of AF64A on water and food consumption are shown in Fig. 1. Statistical analysis of the consumption data was carried out using a repeated measures analysis of variance with the data being broken down by groups (6 nmoles, 3 nmoles, vehicle) and days (3-day baseline, days 1–10, day 21). Analysis of the data for water consumption (top curves) showed that the groups did not differ overall ($p>0.05$), but groups did differ in the amount of water consumed over days, $F(22,132)=13.66$, $p<0.001$. Further analysis of the significant interaction effect with the Neuman-Keuls test indicated that water consumption in the animals that received 6 nmoles was less than that of the vehicle controls for three days but returned to pre-operative levels by day 4. While the analysis of the data for amount of food consumed during the 21-day period did not reveal any statistically significant differences between groups ($p>0.05$), inspection of Fig. 1 suggests that food consumption in the animals that received injections of AF64A was slightly depressed for approximately six days. The data for the number of 1 sec counts spent drinking and eating will not be presented here since the most important differences are reflected in the consumption data described above.

The basic pre- and postoperative activity data consisted of the number of 1 sec periods an animal was active for the 12 hr night and 12 hr day for 5 days before the operation

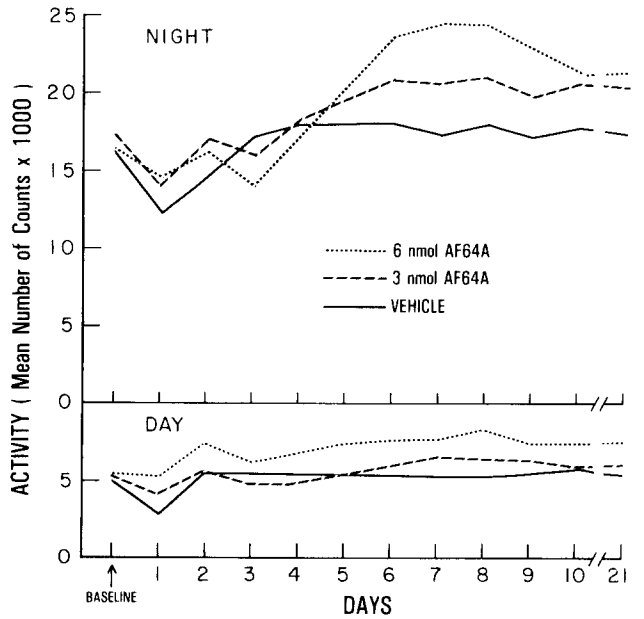

FIG. 2. Mean pre- and postoperative activity for the groups during the night (top) and day (bottom).

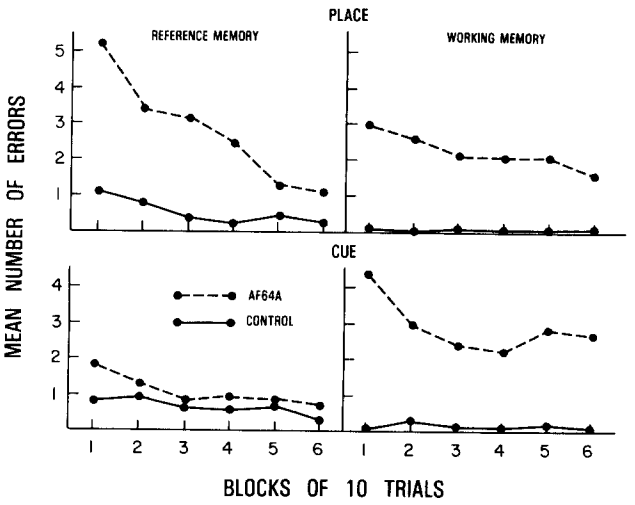

FIG. 3. Mean number of RM (left) and WM (right) errors for the groups on the place (top) and cue (bottom) tasks as a function of blocks of 10 trials during testing for retention.

(baseline), and postoperative days 1–10 and day 21. These activity data are shown in Fig. 2. Statistical analysis of these data indicated that the groups differed overall, $F(2,12)=6.20$, $p<0.01$, and that the animals were more active at night than during the day, $F(1,12)=570.10$, $p<0.01$. The most important finding resulting from analysis of these data was that the groups differed significantly over days, $F(22,132)=1.61$, $p<0.05$. Inspection of Fig. 2 shows that the greatest increases in activity were found in days 6 through 8 in the animals that received 6 nmoles of AF64A. Further analysis of these data using the Neuman-Keuls test indicated that the 6 nmoles group was significantly more active than controls beginning on day 6 and continuing through day 21. While animals in the 3 nmoles group tended to be more active than controls after day 6, the differences were not statistically significant ($p>0.05$).

The reason for the delayed increase in home cage activity following ICV injection of AF64A is not known. However the increase is similar to that previously found in the AED system following interruption of the fimbria and complete aspiration of the hippocampus [12]. In the study involving damage to the septohippocampal system, the increased activity peaked 5 and 6 days following the operation while the largest increases seen in the present study were after 6 to 8 days.

Experiment 2

Two animals in the 6 nmoles group and one in the 3 nmoles group died within three days following the operations. Of the survivors, two in the high dose group and one in the low dose group would not run on the maze when testing was resumed. Since inspection of the behavioral data for the remaining injected animals indicated a similar pattern of performance, data for the subjects remaining in the 6 nmoles group (n=2) and 3 nmoles group (n=4) were pooled for purposes of analysis.

Postoperative performance in the radial maze is shown in Fig. 3 where the error data are broken down by groups (AF64A and control), task (place and cue), type of memory error (WM and RM), and blocks of trials. Working memory errors are repeated entries into correct arms (or cues) that have already been visited during the trial; RM errors are choices of arms or cues that have never been baited. Statistical analysis using a repeated measures analysis of variance of performance on the place task (top curves) indicated that the AF64A-injected animals made more overall errors than controls, $F(1,8)=12.00$, $p<0.001$, but WM was not more affected than RM, $p>0.05$. The most important finding resulting from analysis of the performance on the cue task (bottom curves) was that rats in the treated group did not differ from controls in number of RM errors, but made significantly more WM errors, $F(1,8)=5.70$, $p<0.05$. Thus, animals receiving injections of AF64A made more WM errors than controls on both the place and cue task, but more RM errors only on the place task.

Reversal learning, where the opposite four arms and cues were baited, yielded impairments in performance that were generally similar to those obtained in postoperative retention testing. That is, the AF64A-treated group made more errors than controls on both the place, $F(1,8)=28.96$, $p<0.001$, and cue task, $F(1,8)=4.42$, $p<0.05$.

Histology

Histological examination of cell-stained brain sections of AF64A injected rats revealed extensive damage to the brain at the site of injection. Figure 4 is a photomicrograph of a brain section from a rat that was injected unilaterally on the right with 1.5 nmoles of AF64A and on the left with vehicle. The animal was sacrificed and perfused after 7 days. Figure 5 is a photomicrograph of a coronal section from a bilaterally injected (1.5 nmoles per side) rat in Experiment 2 that was sacrificed 13 weeks after AF64A injection. The damage to

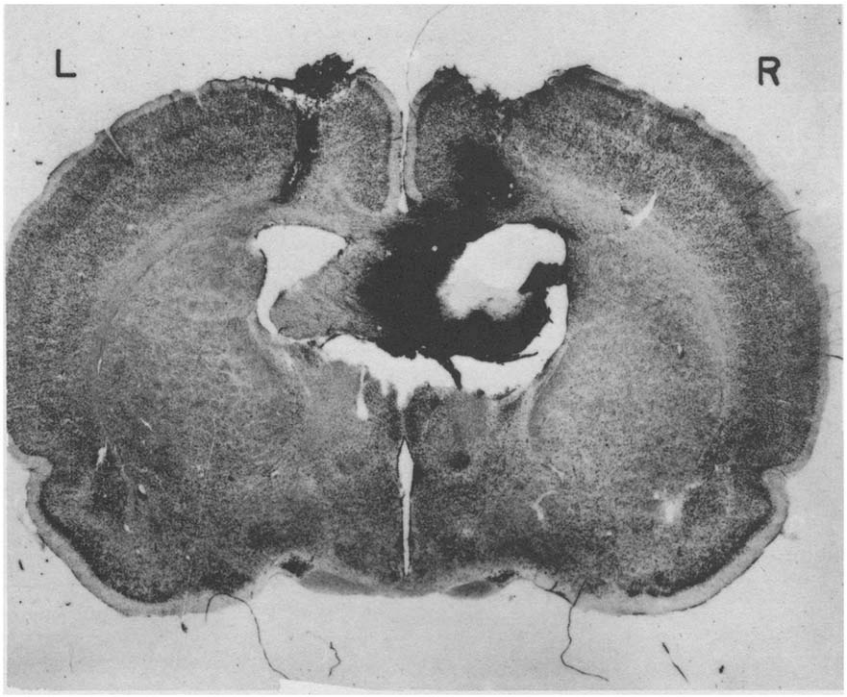

FIG. 4. Photomicrograph of a coronal section at the site of injection showing the resulting brain damage following injection of AF64A on the right side and vehicle on the left side (1.5 nmole, 1 week survival).

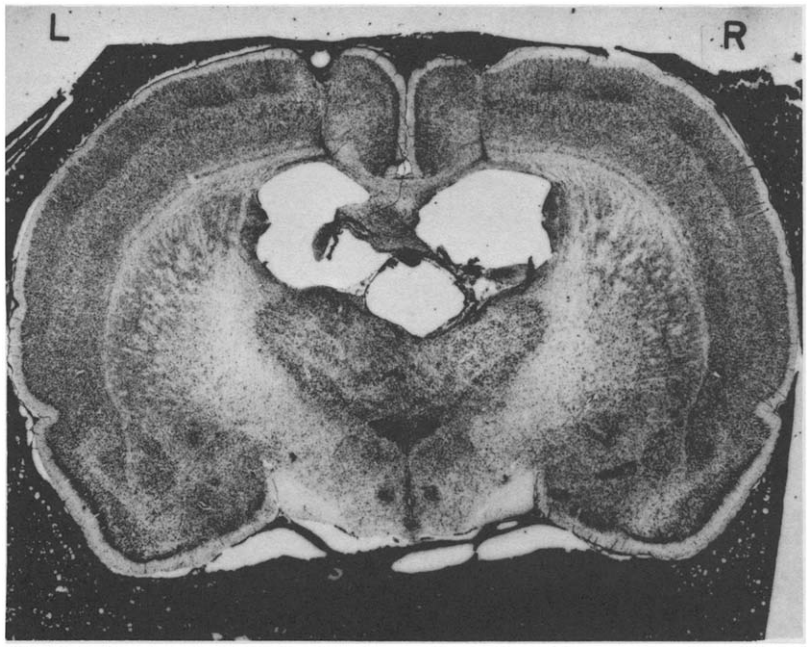

FIG. 5. Photomicrograph of a coronal section at the site of injection following bilateral injection of AF64A (1.5 nmoles each side, 13 week survival).

TABLE 1
ACETYLCHOLINE LEVELS IN AF64A TREATED RATS

Brain Region	Control	Group 1 1 nm/1 μl	Group 2 1 nm/3 μl	Group 3 3 nm/1 μl	Group 4 3 nm/3 μl
Front Cortex	17.0 ± 1.5	16.3 ± 2.4	15.8 ± 0.6	18.0 ± 1.4	15.3 ± 2.2
Striatum	48.0 ± 4.0	34.1 ± 2.2*	30.7 ± 2.1‡	32.6 ± 3.2*	26.9 ± 4.2†
Hippocampus	23.3 ± 1.3	21.7 ± 1.7	16.5 ± 1.2†	16.1 ± 2.6*	11.6 ± 1.2†
Hypothalamus	28.7 ± 2.3	38.2 ± 6.3	29.3 ± 6.3	25.2 ± 3.4	30.4 ± 3.2
N. accumbens	65.4 ± 5.1	53.0 ± 3.9	51.7 ± 4.6	59.5 ± 7.2	45.1 ± 7.8
Septal Area	41.0 ± 2.0	33.2 ± 4.5	33.6 ± 3.7	31.2 ± 4.5	23.4 ± 1.6†

Values represent the mean ± SEM of 6 animals. Differs from control, *$p<0.05$, †$p<0.01$, Student's t-test.

TABLE 2
COMPARISON OF ACETYLCHOLINE AND CATECHOLAMINE DEPLETION BY AF64A

	Striatum		Hippocampus	
	(% control)			
	ACh	DA	ACh	NE
Control	100 ± 8.8	100 ± 4.5	100 ± 5.5	100 ± 12.4
AF64A (1 nmole in 1 μl)	71 ± 4.6†	109 ± 6.7	93 ± 7.5	105 ± 12
AF64A (1 nmole in 3 μl)	64 ± 4.4†	101 ± 5.8	71 ± 5.4†	90 ± 13.9
AF64A (3 nmole in 1 μl)	68 ± 6.6†	110 ± 14	69 ± 11†	93 ± 16
AF64A (3 nmole in 3 μl)	56 ± 8.7†	108 ± 9.0	50 ± 5.2†	75 ± 5.7*

Values represent the mean ± SEM with n=6. Differs from control, *$p<0.05$, †$p<0.01$, Student's t-test.

the fimbria-fornix and the extensive gliosis on the AF64A-injected side in Fig. 4 is readily apparent.

In all animals in the 6 nmoles group, the fimbria-fornix was completely bisected at the level of the injection. Damage to the fimbria-fornix in the 3 nmoles group was less extensive but in every animal at least the lateral third of the fiber bundle was lesioned on both sides. In three of the ten rats in the 3 nmoles group, damage to the fimbria-fornix was completed. Damage resulting from the injection of the compound extended rostrally to include bilateral loss of cells in the lateral septum adjacent to the ventricles in all animals receiving 6 nmoles; bilateral damage to lateral septum was found in four rats in the 3 nmoles group. Several animals in the 6 nmoles group had bilateral loss of cells in the rostral tip of dorsal hippocampus, and in several animals the hippocampal damage was limited to one side. Animals in the 3 nmoles group did not have any apparent damage to hippocampus. The ventricles were enlarged in most animals, especially in those injected with 6 nmoles of the compound. Small areas of necrosis were found in the hypothalamic area surrounding the third ventricle in some animals. There was no apparent cell loss in that part of the caudate-putamen nucleus that borders the lateral ventricles at the site of injection. This might indicate that this region is less sensitive to the toxic effects of the compound. Careful inspection of sections stained with the Fink-Heimer silver stain revealed a pattern of degeneration that was similar to that found following radiofrequency lesions of the fimbria-fornix [13].

Experiment 3

Acetylcholine levels were depleted in several brain regions adjacent to the lateral ventricle as shown in Table 1. Significant depletions were seen in the corpus striatum, hippocampus and septal region while the nucleus accumbens was slightly depleted and the frontal cortex and hypothalamus were not depleted at all. These data confirm the observations of Fisher, Mantione, Abraham and Hanin who also found specific depletions of ACh in the hippocampus and striatum of treated mice [7]. The depletion of ACh was lo-

calized to the regions surrounding the ventricles. The different dose-volume AF64A injection combinations produced differential depletions. Both increased volume and increased dose resulted in greater depletions with the 3 nmoles in 3 μl combination causing the greatest ACh depletion.

As shown in Table 2, the effects of AF64A injection into the lateral ventricle seem to be primarily on cholinergic systems. DA levels in corpus striatum were not affected at any dose-volume tested, although ACh levels were decreased to 56% of control. Also, NE levels in hippocampus, except at the highest tested dose, were unaffected at dose-volume combinations of AF64A that significantly depleted ACh.

GENERAL DISCUSSION

Over the last two decades, neurotoxins have played an increasingly important role in the study of various brain mechanisms. The final goal has always been to use these neurotoxins on specific neuronal systems, in well defined structures of the brain. However, for each neurotoxin the appropriate experimental conditions had to be established to ensure a specific action of the toxin and a clear interpretation of the results.

Injections of AF64A into the lateral ventricles at the level of the fimbria-fornix resulted in extensive behavioral, anatomical and neurochemical changes. Treatments of 6 and 3 nmoles resulted in a loss of 33.3 and 16.7% of the animals, respectively. Thus, even at the relatively low concentrations used in our experiments the compound proved to be quite toxic.

Results obtained in the first experiment (Fig. 1) indicate that, following a post-surgery recovery of four days, water consumption returns to baseline levels while normal food consumption is regained within seven days. Therefore, the performance of rats in the radial maze should not have been affected by differences in appetitive motivation.

The pattern of increases in home cage activity that resulted from ICV injections of the compound were similar to those reported following fimbria and complete aspiration lesions of the hippocampus [12]. Although the activity of our AF64A animals was not tested in a novel exploratory situation, in previous research open-field activity was increased in fimbria and hippocampal lesioned rats [12]. While the present research was in progress, it was reported by Walsh, Tilson, Fisher and Hanin that ICV injection of AF64A reduced motor habituation in a novel situation [27]. The concentration of AF64A employed by these investigators was considerably larger than those used in the present study (30 and 15 nmoles as compared to our 6 and 3 nmoles).

The rats in our experiments that survived the 6 nmoles of AF64A treatment were hyperreactive and extremely difficult to handle. In fact, observations of the animals suggested a pattern of behavior similar to that described in the literature as "the septal syndrome" [2]. Russell and Macri [19] and Russell and Jenden [20] reported that decreased cholinergic activity caused by ICV injections of hemicholinium-3 also results in hyperreactivity. The hemicholinium-induced hyperreactivity correlated with ACh depletion in the hippocampus.

The complex radial maze tasks used in the present study were designed to study two kinds of learning (place and cue) and two memory functions (WM and RM). AF64A-treated rats were impaired in WM on both the place and cue tasks, i.e., the rats repeated correct arms (and cues) that had already been visited within the trial. These results suggest that the treated animals had difficulties holding information that is pertinent only within a short period of time (see [17]). This impairment is identical to that found by the senior author in other research following radiofrequency lesions of the fimbria-fornix and entorhinal cortex [13], but is different from that found following direct damage to the hippocampus [11]. Walsh *et al.* [27] reported that rats receiving ICV injections of 30 and 15 nmoles of AF64A were impaired in a radial arm maze with all arms baited. Since no attempt was made in their research to look at either place vs. cue, or WM vs. RM, little can be said about the nature of the memory impairment experienced by their surviving animals.

Histological examinations of the brains of the rats that received ICV injections of the compound indicated that most axons in the fimbria-fornix were interrupted. Since septal lesions persistently reduce ACh in hippocampus [23], and the cholinergic pathway that projects from the medial septal nucleus to the hippocampus is known to pass through the fimbria-fornix [26], one would expect a decrease in levels of ACh in the hippocampus in the present research. Our neurochemical results (Experiment 3) indicate that ACh levels are depleted by approximately 50% following injection of AF64A into the ventricles adjacent to the fimbria-fornix.

It is not possible in the present research to attribute the observed behavioral and neurochemical changes to the specific effects of AF64A on cholinergic systems of the brain. Although the primary neurochemical change observed was the depletion of acetylcholine, this outcome may have been the result of the anatomical placement of the injection rather than the cholinergic specificity of the neurotoxin injected. Norepinephrine and dopamine were not much affected by the procedure used but other neurotransmitters, such as serotonin, or neuropeptide systems might also have been damaged. While the behavioral changes observed after AF64A appear similar to those seen after hemicholinium treatment or radiofrequency lesions of the fimbria-fornix, we cannot definitively conclude that the behavioral changes seen after AF64A were caused by damage to cholinergic systems alone. In other research designed to test the non-specific toxic effects of the compound, we have found that injections of AF64A into the substantia nigra result in neurochemical, behavioral, and anatomical changes similar to those found following electrolytic lesion of the substantia nigra [15].

Cholinergic specificity of AF64A may depend on a number of experimental conditions including dose and volume injected, the time over which the compound is infused, the site of injection, and possibly the anesthetic employed. For example, it has been reported that sodium pentobarbital decreases brain ACh turnover [9], and appears to decrease choline uptake. It is possible that such an effect could reduce the cholinergic toxicity of AF64A and enhance its non-specific effects. Given the potential usefulness of a specific cholinergic neurotoxin, research into the relevant experimental conditions should be vigorously pursued.

In view of the present results, it would appear that researchers using AF64A should be careful to determine that observed behavioral and/or neurochemical changes cannot be attributed to non-specific effects of the compound.

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