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Hippocampal Injections of Amyloid β-Peptide 1-40 Impair Subsequent One-Trial/Day Reward Learning

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The injection of amyloid β-peptide (Aβ) into rat CNS has been reported to induce cellular neuropathology. The present study investigated whether multiple intrahippocampal injections of Aβ 1-40 would impair one-trial/day reward learning 14 days later. Twenty-four male Sprague–Dawley rats, 3–4 months old, were injected with either Aβ 1-40 or distilled water into seven hippocampal sites bilaterally. Ten rats received 3 nmol Aβ 1-40 in 2 μl of distilled water per injection site, while 14 rats received distilled water alone. Following a 9-day recovery period, rats were gradually food deprived to 82% of their initial body weight. Fourteen days after the intrahippocampal injection, all rats received an initial training trial and three subsequent daily retention trials. Rats receiving Aβ 1-40 were significantly impaired on the second retention trial in terms of accuracy (number of unbaited alleys entered) and on the second and third retention trials in terms of speed (reciprocal of latency to reward). Histological analysis showed that Aβ 1-40 injections produced significant neuronal loss and gliosis. Aβ 1-40 immunoreactivity persisted locally at the injection site and in macrophages 2 weeks following the hippocampal injections. These effects appear to be sequence-specific; rats receiving

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Aβ 1-42 with a scrambled peptide sequence did not differ significantly from rats receiving distilled water alone in retention of the learning task or degree of histological damage.

Key Words: amyloid β-peptides; hippocampus; reward learning; memory impairment; neurodegeneration; Alzheimer's disease; rat.

Amyloid β-peptide (Aβ) is a major constituent of the senile plaques which occur in the brains of Alzheimer's disease (AD) patients (Selkoe, 1994). It has been suggested that insoluble deposits of Aβ are neurotoxic and may contribute to the degenerative changes and cell death observed in AD (Selkoe, 1994; Yankner, Duffy, & Kirschner, 1990). In cell culture, Aβ or its fragments has been reported to be toxic to mature cortical neurons (Weiss, Pike, & Cotman, 1994; Yankner et al., 1990), hippocampal neurons (Mattson, Tomaselli, & Rydel, 1993; Pike, Cummings, & Cotman, 1992; Takadera, Sakura, Mohri, & Hashimoto, 1993; Yankner et al., 1990), neuronally differentiated PC12 cells (Clark & Vulliet, 1993), and microglia (Korotzer, Pike, & Cotman, 1993). Several other studies found that while Aβ was not toxic by itself, it was toxic in combination with excitatory amino acids or calcium iontophores (Koh, Yang, & Cotman, 1990; Mattson, Cheng, Davis, Bryant, Lieberburg, & Ryder, 1992). *In vivo* injection of Aβ 1-40 into rat cortex and hippocampus has been reported to result 7 days later in signs of Alzheimer-like neuropathology (Kowall, Beal, Busciglio, Duffy, & Yankner, 1991; Kowall, McKee, Yankner, & Beal, 1992). Aβ 1-40 also induced local neurodegeneration when injected into hippocampal slice cultures (Malouf, 1992). Injection of the Aβ-rich cores of senile plaques from AD patients induced local pathology in rat brain (Frautschy, Baird, & Cole, 1991). However, injection of synthetic Aβ peptides into brain tissue has sometimes failed to result in tissue damage (Clemens & Stephenson, 1992; Games, Khan, Soriano, Keim, Davis, Bryant, & Lieberburg, 1992; Podlisny, Stephenson, Frosch, Lieberburg, Clemens, & Selkoe, 1992; Stein-Behrens, Adams, & Sapolsky, 1992). It has been suggested that variations in the aggregation state of Aβ peptides may determine their degree of toxicity and account for these discrepant results (Pike, Walencewicz, Glabe, & Cotman, 1991; Snow & Malouf, 1993).

The inability to retain new information is a major behavioral symptom of AD (particularly in the earlier stages). This raises the following question: can Aβ injection result in sufficient neural degeneration to cause measurable impairment of learning and memory? Immediate posttrial injection of Aβ 1-40 (intrahippocampal) or several Aβ fragments (icv) interfered with subsequent retention of avoidance (Flood, Morley, & Roberts, 1991) and spatial (McDonald, Dahl, & Overmier, 1994) learning. Rats were also impaired in spatial learning during continuous icv infusion of Aβ 1-40 (Nitta, Itoh, Hasegawa, & Nabeshima, 1994). However, these results might conceivably be interpreted as immediate or acute pharmacological effects of Aβ, as opposed to long-term degenerative consequences, since the subjects were still receiving Aβ during learning and/or memory consolidation. The question thus remains, does tissue deposition of Aβ trigger sufficient long-term neural degeneration to impair subsequent memory formation weeks later? Intrahippocampal injections of the Aβ 25-35 fragment combined with the excitotoxin ibotenic acid resulted in impaired spatial learning 8–10 days later (Dornan, Kang, McCampbell, & Kang, 1993). There have been very few published studies of the long-term behavioral effects of Aβ peptides by themselves. Injection of 10 μg of Aβ peptides into the nucleus

basalis disrupted object recognition performance during the first weeks after injection in the case of Aβ 25-35, but only beginning 2 months after the lesion in the case of Aβ 1-40 (Giovannelli, Casamenti, Scali, Bartolini, & Pepeu, 1995). Injection of Aβ 25-35, Aβ 1-40, and Aβ 1-42 resulted 4, 7, and 14 days later in significant impairment on a rat social recognition task, employed as a model of working memory (Terranova, Kan, Storme, Perreaut, Le Fur, & Soubrié, 1996).

The current study determined whether multiple, much smaller, hippocampal microinjections of Aβ 1-40 would impair one-trial per day discriminative reward learning 14 days later. One-trial per day discriminative reward learning using a five-alley sunburst maze is a training paradigm introduced by Malin, Toups, Osgood, Fowler, Hunter, Arcangeli, and Moss (1991a). This task is designed to separate learning from performance effects by comparing retention trial performance with baseline training trial performace and by counting errors (numbers of entries into the four unbaited alleys) in addition to measuring latency to reward. A highly energetic and motivated animal might achieve a short latency by performance factors alone, but would be unlikely to avoid errors without learning, particularly since the baited alley requires climbing and thus has a higher response cost. An age-related retention deficit has been demonstrated on this task: 2- to 3-month-old rats retain this task readily 1 day after a single training trial, while 18- to 24-month-old rats show very little retention (Malin et al., 1991a; Malin, Plotner, Radulescu, Ferebee, Lake, Negrete, Schaefer, Crothers, & Moss, 1993).

Degeneration of forebrain cholinergic pathways is a critical factor in AD (Bartus, Dean, Beer, & Lippa, 1982), and intracerebral Aβ has toxic effects on brain cholinergic systems (Abe, Casamenti, Giovannelli, Scali, & Pepeu, 1994; Emre, Geula, Ransil, & Mesulam, 1992; Giovannelli et al., 1995). Therefore, it is relevant that retention of the one-trial discriminative reward task appears to be dependent on cholinergic mechanisms. Impaired retention in 18-month-old (Malin et al., 1991a) and 24-month-old (Malin et al., 1993) rats was effectively reversed by pretraining treatment with the CNS-selective cholinesterase inhibitor methanesulfonyl fluoride. Conversely, icv injection of galanin, a negative modulator of acetylcholine, prior to the single training trial, largely prevented subsequent retention (Malin, Novy, Lett-Brown, Plotner, May, Radulescu, Crothers, Osgood, & Lake, 1991b).

In the current study, Aβ 1-40 was injected bilaterally into multiple hippocampal sites. The hippocampus was chosen because it plays a major role in learning and memory formation (Damasio, Eslinger, Damasio, Van Hoesen, & Cornell, 1985; Zola-Morgan, Squire, & Amaral, 1986). One potential difficulty is that the hippocampus is more clearly involved in working as opposed to reference memory (Walker & Olton, 1984). One-trial discriminative reward learning might possibly be construed as a reference memory task, since the information to be retained is not varied from trial to trial as in working memory procedures. However, in monkeys, one-trial per day discrimination learning (the same basic learning paradigm employed in the present experiments) depends on intact hippocampal and amygdala circuits for the first few days, despite the fact that this is a reference memory task (Malamut, Saunders, & Mishkin, 1984). In addition, there seems to be a large component of place learning in the one-trial reward task. Scrambling the location of the baited alley (without changing its appearance) results in a major loss of retention (unpublished). Hippocampal lesions have been shown to devastate pure place reference learning (Schenk & Morris, 1985), although the interpretation of this effect is complex (Eichenbaum, Stewart, & Morris, 1990). Also, the hippocampus is one of the brain regions

with the highest concentration of amyloid-containing senile plaques in AD (Braak & Braak, 1991; Hyman, Van Hoesen, & Damasio, 1990). As a control for nonspecific effects (Podlisny et al., 1992), a second experiment tested the effect of Aβ 1-42 with a scrambled sequence (containing all of the amino acid residues found in Aβ 1-40 plus additional copies of two amino acids).

EXPERIMENT 1: INTRAHIPPOCAMPAL Aβ 1-40 IMPAIRS ONE-TRIAL PER DAY REWARD LEARNING 14 DAYS LATER

Methods

Subjects

The subjects were 24 male Sprague–Dawley rats, 3–4 months old, weighing 400–540 g. Subjects were handled daily (19 days) to habituate them to human contact. Each rat was habituated to the maze for 3 min on the day prior to brain injections.

Brain Injections

Injections of Aβ 1-40 (Bachem, Torrance, CA) or distilled water alone were performed under equithesin anesthesia and stereotaxic guidance. Each rat received microinjections into seven hippocampal sites bilaterally, all within a single coronal plane. Two sites were in the dorsal hippocampus (3.85 mm posterior, 3.60 mm lateral, 3.8 and 5.5 mm inferior to bregma) and five were in the ventral hippocampus (3.85 mm posterior, 5.00 mm lateral, 5.2, 6.3, 7.5, 8.6, and 9.8 mm inferior to bregma). Ten rats received 3 nmol Aβ 1-40 in 2 μl of distilled water per injection site, while 14 rats received distilled water alone. The dose per site was the same previously shown to induce roughly a 2-mm-diameter area of neurodegeneration in rat hippocampus (Kowall et al., 1991). The pattern of injection sites was chosen so that 2-mm zones of degeneration would disrupt a considerable proportion of cross-hippocampal circuits. Distilled water was used as the solvent since saline impaired the solubility of Aβ 1-40. All injections were gradually infused at a rate of 4 μl/min using a motorized syringe, with a 1-min pause after completion of the injection for absorption at each site.

Behavioral Apparatus

A black Plexiglas sunburst maze (Malin et al., 1991a) containing a start box and five alleys radiating from a central choice point was used. Four alleys were level and unbaited; the single baited alley had a grid floor that ascended at 45°. Ten Bioserve reward pellets (45 mg each) were placed in a recessed cup at the top of the ascending alley.

Training/Testing Procedures

Nine days were allowed for recovery from stereotaxic brain surgery. Rats were then gradually food deprived to approximately 82% of their initial body weight over a period of 5 days. Thus, the initial training trial was conducted 14 days following intrahippocampal injections. This interval was chosen to allow time for clearance of soluble Aβ peptide

and for degeneration of neurons, which occurred in previous studies by 1 week after Aβ injection (Kowall et al., 1991, 1992) or 4 weeks after senile plaque core injection (Frautschy et al., 1991). After 30 s in the start box, each rat was allowed to wander freely in the maze. Each rat was observed for number of errors (blind alleys entered) and latency to reach reward. As customary in discrete trial learning, promptness in reaching the reward (arbitrarily designated by the term "speed") was expressed in terms of the reciprocal of latency, so that a single slow, sick, or unmotivated subject with an extremely long latency will not have a disproportionate effect on the group average. All speed scores were multiplied by 100 so that speed data would not be inconveniently expressed in small decimal fractions. Subjects were retested in the same manner at 24-h intervals for 3 days. Retention scores were determined as the decrease in errors or the increase in speed from the initial training trial. All observations were carried out under "blind" conditions.

Histology

Following the behavioral experiment, rats were sacrificed by decapitation and the brains were removed and stored in 10% formalin, pH 7.4, at 8°C. After fixation, the brains were cryoprotected in 20% glycerol–2% dimethylsulfoxide in phosphate buffer at 4°C. Brains were blocked, and serial 35-μm-thick frozen sections cut on a sledge microtome were collected sequentially and without interruption into six wells. Sections from one well (every sixth section) were mounted and stained with cresyl violet as a general cell stain. Selected sections from regions containing the hippocampus were processed for Aβ immunoreactivity. These sections were treated for 30 min in 0.3% hydrogen peroxide in absolute methanol followed by several washes with cold phosphate-buffered saline (PBS, 0.1 M, pH 7.3). Following incubation in 10% normal goat serum in PBS, sections were placed in primary Aβ antibody (courtesy of Dr. B. Yankner) diluted 1:1000 in 5% normal goat serum and 0.3% Triton X-100 at room temperature. After overnight incubation on a rocker, sections were washed several times in PBS and incubated for 3 h in peroxidase-conjugated secondary antibody (Boehringer Mannheim, Indianapolis, IN) diluted 1:200 in PBS. After further PBS washes, sections were incubated in 0.05% diaminobenzidine tetrahydrochloride–0.005% hydrogen peroxide in TRIS–HCl buffer, pH 7.5, and monitored by intermittent microscope examination. The peroxidase reaction was terminated by washes in PBS. Sections were mounted, air dried, and coverslipped.

Semi-Quantitative Estimation of Lesion Extent

One complete set of serial sections was mounted and stained with cresyl violet. Lesions were examined blindly using a calibrated eye piece graticulate. Contiguous 0.16-mm^2 areas (9400 μm^2) of hippocampus were examined at 200× to determine the maximal extent of the lesion. Lesion severity was graded from 0 to 3 using a semi-quantitative rating scale as follows:

0, needle track and injection site seen but no definite surrounding damage;

1, definite damage surrounding the injection site with inflammatory cells, neuronal loss, and gliosis extending up to 400 μm (graticulate size of one field at 200×) around the injection site with no tissue loss;

2, definite damage surrounding the injection site with inflammatory cells, neuronal

loss, and gliosis extending more then 400 μm but less than 1200 μm (three fields at 200×) around the injection site; There may be minimal necrosis at the injection site;

3, localized necrosis with extensive inflammatory infiltrate, neuronal loss, and gliosis extending more than 1200 μm around the injection site.

Results

Accuracy

Figure 1 shows accuracy retention scores (decrease in errors from initial training trial) over three retention trials in rats pretreated hippocampally with Aβ 1-40 in distilled water or with distilled water alone. The control rats approached perfect performance by the second retention trial, committing 0.36 ± 0.13 errors (M ± SEM), while the Aβ-treated rats still committed 1.5 ± 0.31 errors. ANOVA with one repeated measures variable revealed no significant Drug Effect (Aβ 1-40 vs vehicle), $F(1, 22) = 0.55$, NS. The Trial Effect was significant, $F(2, 44) = 9.81$, $p < .01$. The Interaction Effect (Drug × Trial) was also significant, $F(2, 44) = 3.18$, $p < .05$. Post hoc analysis (Tukey's HSD) revealed a significant difference between the Aβ and vehicle groups on the second retention trial, $p < .01$. The Aβ injections did not impair performance on the initial training trial (prior to memory formation). Baseline errors were 2.50 ± 0.86 in the Aβ group and 3.71 ± 1.61 in the vehicle control group. This difference was not significant, $t(22) = .59$, NS.

Promptness to Reward (Speed)

Figure 2 shows speed retention scores (increase in speed as measured by 100 × reciprocal of latency) from the initial training trial over three retention trials in rats pretreated hippocampally with Aβ 1-40 in distilled water or with distilled water alone. These scores were subjected to ANOVA with one repeated measures variable. The Drug Effect (Aβ vs vehicle) approached significance, $F(1, 22) = 4.01$, $.05 < p < .10$. The Trial Effect was significant, $F(2, 44) = 18.73$, $p < .01$. The Interaction Effect (Drug × Trial) was also significant, $F(2, 44) = 3.82$, $p < .05$. Post hoc analysis (Tukey's HSD) revealed significant differences between the Aβ and vehicle groups on the second and

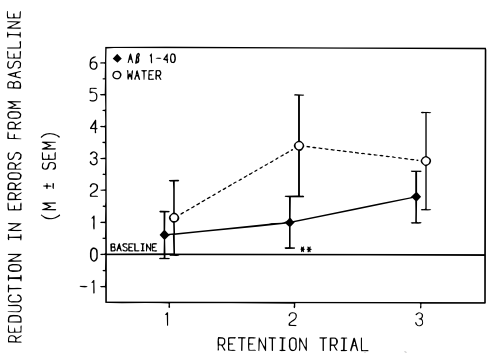

FIG. 1. Accuracy retention scores (reduction in errors from initial training trial on three daily retention trials). Black diamonds: 10 rats receiving seven injections into each hippocampus of 3 nmol Aβ 1-40 in 2 μl of distilled water. Open circles: 14 rats receiving equivalent injections of distilled water alone. ** $p < .01$ vs distilled water.

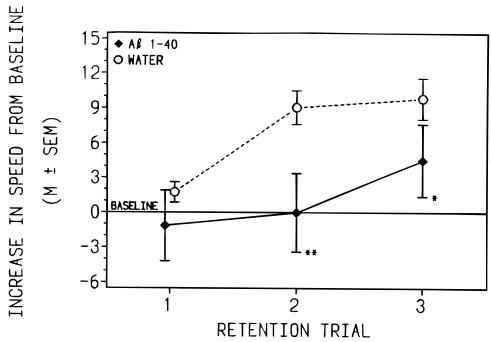

FIG. 2. Speed retention scores (increase in speed from initial training trial on three daily retention trials). Speed is measured by 100 s × reciprocal of latency to reward. Black diamonds: 10 rats receiving seven injections into each hippocampus of 3 nmol Aβ 1-40 in 2 μl of distilled water. Open circles: 14 rats receiving equivalent injections of distilled water alone. * $p < .05$, ** $p < .01$ vs distilled water.

third retention trials, $p < .01$ and $p < .05$, respectively. The Aβ injections did not impair performance on the initial training trial. Speed scores were 6.61 ± 3.11 in the Aβ group and 2.72 ± 0.40 in the vehicle control group. This difference was not significant, $t(22) = 1.47$, NS.

Histology

Aβ lesions were characterized by local neuronal loss and gliosis that typically extended from 300 to 900 μm around the injection site (Fig. 3). In some cases, localized necrosis was evident. The median lesion score on a 0–3 scale was 1.75 for the Aβ group compared to a median score of 0.5 for the controls. The normal approximation of the nonparametric Wilcoxon test (Marasculio & Sweeney, 1977) indicated that lesion scores of Aβ-injected rats ranked significantly higher than those of vehicle-injected controls, $Z = 1.96$, $p = .025$. In most cases, residual Aβ deposition was evident in the region of the injection site despite the passage of 17 days between injection and sacrifice (Figs. 3D–3F). The Aβ-immunoreactive deposits were either free in tissue or within macrophages seen some distance from the injection site.

Relationship between Histological Changes and Retention Deficits

In order to test the hypothesis that the Aβ-induced tissue degeneration mediated the observed memory impairments, each rat's lesion score was correlated with measures of task retention. Lesion scores of all subjects correlated negatively, $r = -.282$, with retention in terms of accuracy (reduction in errors from baseline to the second retention trial). This correlation approached significance, $.05 < p < .10$. Lesion scores also correlated negatively, $r = -.412$, with retention in terms of speed (speed increases from baseline to the second retention trial). This correlation was significant, $p < .05$.

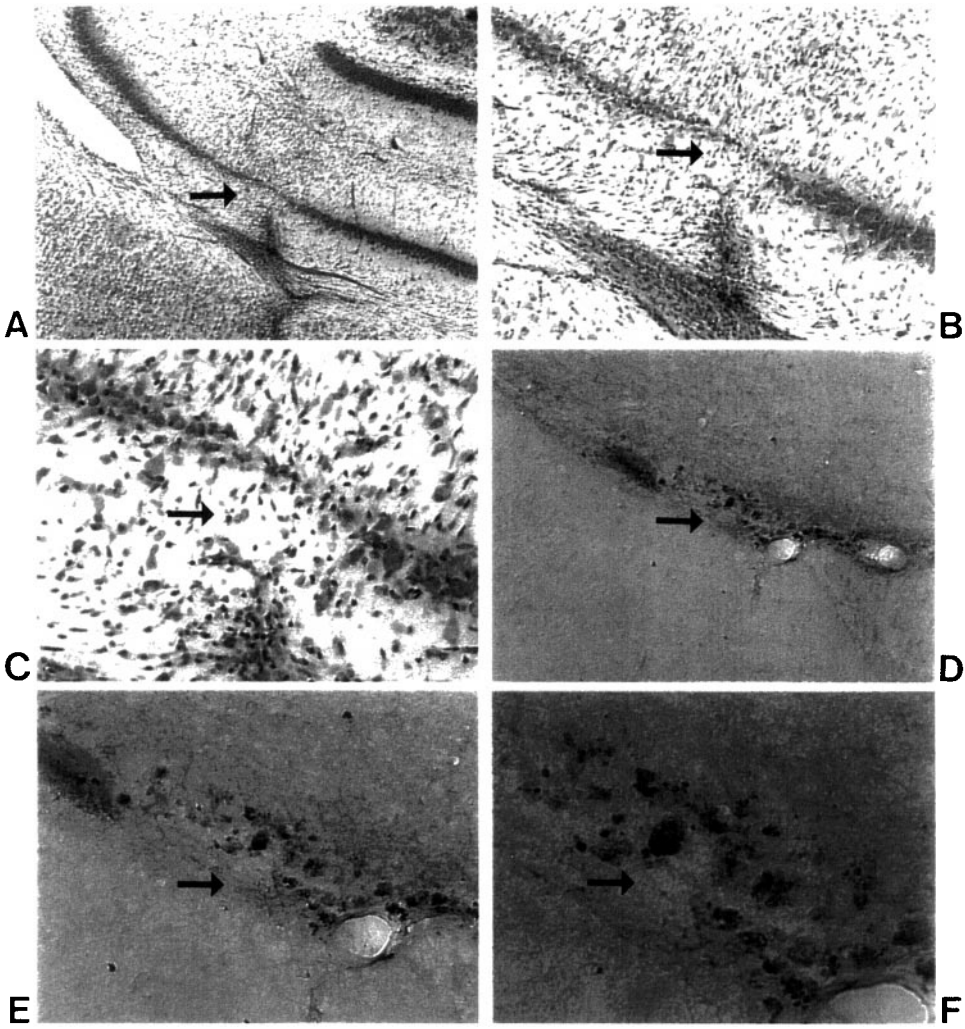

FIG. 3. Histological analysis of Aβ 1-40 injections with cresyl violet (A–C) and Aβ immunoperoxidase (D–F) stains (from a brain receiving a lesion score of 3 based on multiple sections). A: Low-power overview of the hippocampal injection site. The pyramidal layer of CA1 is interrupted and gliosis is evident (arrow); original magnification 40×. B: Higher power view of the same lesion shows the neuronal loss in CA1 (arrow), original magnification 100×. C: High-power view shows gliosis and neuronal loss (arrow), original magnification 200×. D: Aβ immunoreactive deposits in the hippocampus (arrow); original magnification 200×. E: Aβ immunoreactive deposits are irregular and globular. Some appear to be intracellular (arrow); original magnification 200×. F: High-power view shows macrophages containing Aβ immunoreactivity (arrow); original magnification 400×.

EXPERIMENT 2: INTRAHIPPOCAMPAL SCRAMBLED Aβ 1-42 DOES NOT IMPAIR ONE-TRIAL PER DAY REWARD LEARNING 14 DAYS LATER

Methods

The methods were identical to those of Experiment 1, except that rats received 14 intrahippocampal injections of either distilled water alone or scrambled sequence Aβ 1-

42 (KVKGLIDGAHIGDLVYEFMDSNSAIFREGVGAGHVHVAQVEF), generously donated by Abbott Laboratories. This water-soluble compound was used in preference to the reverse peptide Aβ 40-1 which demonstrates considerable neurotoxicity and aggregation (Emre et al., 1992; Giordano, Bao Pan, Monteggia, Holzman, Snyder, Krafft, Ghanbari, & Kowall, 1994, McKee, Kowall, Schumacher, & Beal, 1998). Unfortunately, scrambled Aβ 1-40 was not available in sufficient quantities for multiple brain injections. Eight rats received 3 nmol scrambled Aβ 1-42 in 2 μl of distilled water per injection site, while another eight rats received equivalent injections of distilled water alone.

Results

Scrambled Aβ 1-42 had very little effect on subsequent one-trial reward learning. Figure 4 shows accuracy retention scores (decrease in errors from initial training trial) over three retention trials in rats pretreated intrahippocampally with scrambled Aβ 1-42 in distilled water or with distilled water alone. ANOVA with one repeated measures variable revealed no significant Drug Effect (Scrambled Aβ 1-42 vs vehicle), $F(1, 14) = 0.01$, NS. The Trial Effect was significant, $F(2, 28)=7.17$, $p < .01$. The Interaction Effect (Drug $\times$ Trial) was not significant, $F(2, 28) = 0.00$, NS. Post hoc analysis (Tukey's HSD) revealed no significant differences between the scrambled Aβ 1-42 and vehicle groups on any retention trial. Scrambled Aβ 1-42 injection also had little effect on accuracy during the initial training trial. Baseline errors were 4.75 $\pm$ 1.66 in the scrambled Aβ 1-42 group and 4.125 $\pm$ 1.03 in the vehicle control group. This difference was not significant, $t(14) = .32$, NS.

Figure 5 shows speed retention scores (increase in speed from initial training trial) over three retention trials in rats pretreated intrahippocampally with scrambled Aβ 1-42 in distilled water or with distilled water alone. ANOVA with one repeated measures variable revealed no significant Drug Effect (Scrambled Aβ vs vehicle), $F(1, 14) = 0.20$, NS. The Trial Effect was significant, $F(2, 28) = 12.16$, $p < .01$. The Interaction Effect (Drug $\times$ Trial) was not significant, $F(2, 28) = 0.47$, NS. Post hoc analysis (Tukey's HSD) revealed no significant difference between the scrambled Aβ 1-42 and vehicle groups on any retention trial. Scrambled Aβ 1-42 injection also had little effect on speed during the initial training trial. Baseline speed was 2.85 $\pm$ 0.89 in the scrambled Aβ 1-42 group

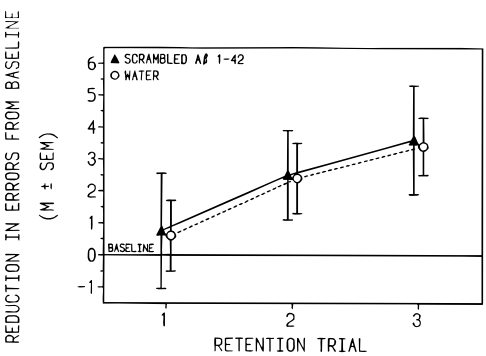

FIG. 4. Accuracy retention scores (reduction in errors from initial training trial on three daily retention trials). Black triangles: eight rats receiving seven injections into each hippocampus of 3 nmol scrambled Aβ 1-42 in 2 μl of distilled water. Open circles: eight rats receiving equivalent injections of distilled water alone.

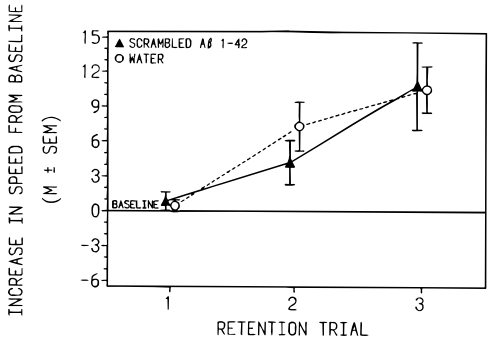

FIG. 5. Speed retention scores (increase in speed from initial training trial on three daily retention trials). Speed is 100 s × reciprocal of latency to reward. Black triangles: eight rats receiving seven injections into each hippocampus of 3 nmol scrambled Aβ 1-42 in 2 μl of distilled water. Open circles: eight rats receiving equivalent injections of distilled water alone.

and 2.29 ± 0.45 in the vehicle control group. This difference was not significant, $t(14) = .56$, NS.

Very little histological damage was detected in brains from rats injected with either scrambled Aβ 1-42 or distilled water alone. The median lesion score was zero (minimal damage) in each group. According to the Wilcoxon test, there was no significant difference in the ranked scores of the two groups, $T(7, 8)=59$, $p = .09$. The normal approximation to the Wilcoxon test could not be used in this case because of a sample size less than 10 (Marasculio & Sweeney, 1977).

GENERAL DISCUSSION

Microinjected Aβ 1-40 persisted in hippocampal tissue for over 2 weeks. As shown in Fig. 3**,** the amyloid deposits were surrounded by areas of cell loss and inflammatory-like infiltration of lymphocytes and astroglia. This picture is generally consistent with that seen 1 week after brain tissue injection of Aβ 1-40 in earlier studies (Kowall et al., 1991, 1992). In the present study, however, distilled water rather than acetonitrile served as the solvent, thus eliminating the possibility that acetonitrile was a necessary cofactor for producing the toxic effects of Aβ 1-40, as previously suggested (Waite, Cole, Frautschy, Connor, & Thal, 1992). The degenerative changes resulting over 2 weeks following seven Aβ 1-40 injections into each hippocampus were sufficient to cause significant retention impairments in both accuracy and speed on certain days of one-trial per day reward learning. The memory impairments were apparently mediated, at least in large part, by these degenerative histological changes, since they were correlated with the lesion scores.

It is not surprising that there were no significant differences on the first retention trial since the control rats showed very little retention on either measure. Thus, there was little room to demonstrate any impairment resulting from Aβ. It was also not surprising that there was no significant difference in errors on the third retention trial, since the control group had already achieved near-perfect accuracy on the second retention trial and had almost no room to improve. The ability of Aβ 1-40 injected rats to efficiently perform the maze task on a delayed basis shows that they were not lacking the basic sensory or motor abilities or activity levels necessary to perform the task. These results are also

reminiscent of an earlier study which found that intact hippocampal/amygdala circuits were essential only to the first several days of one-trial per day discrimination learning (Malamut et al., 1984). The present data suggest that hippocampal function is needed for at least certain stages of learning the one-trial per day discriminative reward task in the rat.

The histological and behavioral impairments seem to be selective to the Aβ peptide sequence, since Aβ 1-42 with a scrambled amino acid sequence had no significant histopathological or behavioral effect. One reservation is that scrambled Aβ 1-40 would have been an ideal control peptide. However, in the absence of sufficient material for multiple *in vivo* administrations, scrambled Aβ 1-42 might be seen as a reasonable control for the amino acid content of Aβ 1-40. It contains all of the amino acids present in Aβ 1-40 plus one additional copy each of isoleucine and alanine. Of course, it has an identical amino acid content to Aβ 1-42, which has also been shown to be toxic to glia and hippocampal neurons (Hoffman, Bi, Pham, & Lynch, 1998; Hu, Akama, Krafft, Chromy, & Eldik, 1998).

The overall results of the present study are consistent with the hypothesis (Selkoe, 1994; Yankner et al., 1990) that brain amyloid deposits may be a causal factor in the neurodegenerative changes and memory impairment associated with AD.

Several previous studies (Flood et al., 1991; McDonald et al., 1994; Nitta et al., 1994) reported that various Aβ peptides impaired subsequent retention of learning when they were administered during or immediately after the learning trials. However, Aβ peptides injected in the hippocampus (current study) as well as the nucleus basalis (Giovannelli et al., 1995) also impaired learning and memory ability weeks later. This prolonged effect of amyloid injections increases the likelihood that the resulting impairments were the result of long-term degenerative changes in brain tissue, as opposed to any immediate pharmacological effects. The present procedure thus more closely models the impairments due to cell loss in chronic degenerative disorders such as AD.

There are, of course, numerous differences between this model and spontaneous amyloid deposition in AD. The amyloid-rich plaques in AD are much smaller and more numerous, resulting in more thorough coverage of the affected areas. They persist for much longer durations than 2 weeks. They occur in many additional brain regions, including the entorhinal cortex, the amygdala, and association cortex (Hyman et al., 1990). Despite these differences, it is hoped that the procedure reported here may be useful for exploring mechanisms of amyloid-induced memory pathology. For example, it might be employed to explore the relative memory pathology resulting from different forms of Aβ, different aggregation states of the peptides, and different proposed cofactors in Aβ toxicity. This procedure might also be useful for preliminary screening of potential therapeutic interventions designed to limit neurodegenerative reactions to Aβ or to compensate for Aβ-induced damage to memory formation pathways.

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