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The Effects of Multiple Injections of BA\((25-35)\) into the Medial Septal Area on Spatial Learning in the Male Rat

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The Effects of Multiple Injections of βA (25-35) into the Medial Septal Area on Spatial Learning in the Male Rat

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Abstract

Accumulating evidence supports the hypothesis that beta-amyloid (βA) plays a role in the degeneration of cholinergic neurons in the basal forebrain. Degeneration of the basal forebrain cholinergic system, which is implicated in learning and memory function, is a characteristic of Alzheimer's disease. It has been previously reported that injections of βA(25-35) into the medial septal area (MSA) of rats produce a hypofunction of the cholinergic system and a decrease in ACh release in the hippocampus. Investigators report mixed results in the development of behavioral spatial learning impairments following single injections of βA. Recent studies suggest that multiple injections or chronic administration of βA results in more consistent spatial learning impairments. One such study recently presented at a neuroscience symposium in Chicago further suggests behavioral deficits in spatial learning ability as a result of a more extensive lesion following the administration of multiple injections of βA(25-35) into the medial septal area. This study replicates and extends this experiment. Injections of 5nm of βA(25-35) were administered into three different levels of the MSA in the male rat and following these injections their spatial learning ability was tested on the Morris water maze and radial arm maze. No significant spatial learning deficits were noted in animals receiving injections of βA(25-35) when compared to controls.
The Effects of Multiple Injections of βA(25-35) into the Medial Septal Area

On Spatial Learning in the Male Rat

Millions of people are currently afflicted with Alzheimer's disease, which is a neurodegenerative disease of aging characterized by a progressive loss of memory. Some of the neuropathological hallmarks of Alzheimer's disease are neuronal death within the basal forebrain accompanied by the appearance of neurofibrillary tangles and neuritic plaques. The major component of neuritic plaques found in the brains of Alzheimer's patients has been identified as a peptide known as beta-amyloid (βA) (for review see Robakis, 1994). One hypothesis of the etiology of Alzheimer's disease (AD) is that aggregation of βA into neuritic plaques leads to selective cell death, resulting in the formation of the behavioral deficits found in AD. Indeed, investigation of the properties and role of βA has provided support for a causal role of βA in the neurodegeneration characteristic in the development of AD (for review see Cotman & Pike, 1994).

In vivo toxicity of two different βA fragments, βA (1-40) and βA (25-35), has been found following injections into the cerebral cortex of rats and monkeys, resulting in lesions larger than those caused by control peptides (Kowall, McKee, Yanker, & Beal, 1992). In aged primate cortex βA (1-40) lesion size has been found to be dose-dependent, suggesting that βA fragments are acutely neurotoxic (Kowall, et al.). The presence of aggregations of βA and neural atrophy within the same areas of the brain indicate that βA may be responsible for the neuronal degeneration found in patients with AD. Results of numerous studies have demonstrated toxic effects of βA and its fragments in areas of the brain involved in memory (Abe, Casamenti, Giovanelli, Scali, & Pepeu, 1994; Harkany, Lengyl et al., 1995; Flood, Morley, & Roberts, 1994).

One particular pathway of the brain implicated in learning and memory affected by βA is the
basal forebrain cholinergic system (for review see Guela & Mesulam, 1994) which includes the cholinergic projections of the nucleus basalis magnocellularis (nBM) to the cortex and amygdala, and the projections of the medial septal area (MSA) to the hippocampus and the parietal cortex. Injections of βA peptide (1-42) into the nBM have been shown to reduce acetylcholinesterase (AChE) and choline acetyl transferase (ChAT) activity as well as significantly reduce the number of muscarinic ACh receptors (Harkany, Lengyl, et al., 1995). Staining also revealed a degeneration of the neural fibers connecting the nBM to the frontal cortex, supporting the toxicity of βA within this cholinergic memory pathway (Harkany, Lengyl, et al.). Other evidence supports the toxicity of βA within other areas of the basal forebrain cholinergic system. Injections of three different βA fragments into the MSA of separate groups of animals have been shown to produce a marked decrease in basal and K+-evoked ACh release in the hippocampus 7 days following injections of 5nm of βA(12-28) and βA(25-35), or 21 days following 3nm of βA(1-40) (Abe et al., 1994).

Collectively, these studies support the idea that βA targets cholinergic neurons, causing hypofunction of the cholinergic system. Approximately 90% of the cholinergic neurons in the brain are found within the basal forebrain cholinergic system, most specifically in the nBM and MSA. Aggregation of βA in these areas along with studies of the toxicity of this peptide and its fragments in these areas, suggest the hypothesis that βA targets these major cholinergic pathways, such as the nBM cortical projection and the septo-hippocampal pathway, which play a role in memory function(Abe et al., 1994; Harkany, Lengyl, et al., 1995).

Further study has supported toxicity of βA to noradrenergic neurons (Emre, Geula, Ransil, & Mesulam, 1992). Intracerebral injections of βA(1-40), when compared to controls injected with the reverse amino acid sequence, revealed significant neurotoxic effects in the immediate vicinity of the injection site on both cholinergic and noradrenergic neurons to relatively the same extent (Emre et al.).
Effects of Multiple Injections of βA (25-35)

Like acetylcholine, norepinephrine (NE) has also been shown to play a role in memory, especially in the neuronal projection from the pontine nucleus locus coeruleus (LC) to the hippocampus. This projection has been shown to be degenerated in patients with AD (Wang, Lui, Reddy, & Barnes, 1994). Administration of βA into the hippocampus is followed by degeneration of NE axons which project to the hippocampus (Wang et al.). Two or three injections of βA (1-40) or βA (25-35), into the hippocampus once a week over three weeks resulted in loss in the hippocampus of neurons containing both glutamate (which is co-localized with NE in the LC) and tyrosine hydroxylase (the rate limiting enzyme in NE synthesis)(Wang et al.).

In contrast to neurotoxic effects of βA on cholinergic and noradrenergic systems within the brain, βA is not toxic to GABAergic neurons located within the basal forebrain cholinergic system (Harkany, DeJong, et al., 1995). These GABAergic neurons within the basal forebrain cholinergic system are not involved in memory function (Harkany, DeJong, et al.). Evidence from these studies assessing the differential toxicity of βA on cholinergic, noradrenergic and GABAergic pathways in the brain (Wang et al., 1994; Harkany, DeJong et al.; Harkany, Lengyl et al., 1995) suggests that βA may be selectively neurotoxic within the neural pathways and transmitter systems involved in memory while not affecting other transmitter systems in the brain not involved in memory function. This evidence is consistent with the hypothesis that βA is the toxic agent responsible for the specific neural degeneration of regions and transmitter systems involved in memory functioning.

While evidence supports the presence of neuropathology following injections of βA which corresponds to that observed in AD patients, conflicting data are reported in the literature on the development of behavioral memory deficits as a result of βA injections. Dornan, D. Kang, McCampbell, and E.Kang (1993) reported that bilateral injections of either two doses of βA(25-35) or a subthreshold dose of ibotenic acid (IBO), an excitotoxin, into the hippocampus failed to produce a
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significant effect on spatial learning in the rat, but identical injections of βA(25-35) and IBO together resulted in a disruption in acquisition of spatial learning when compared to controls (Doman et al.). While no behavioral learning and memory impairments were found following injections of βA or IBO alone, the observed synergistic effects of these βA injections with excitotoxins suggests that neuropathological damage may have been present, but not extensive enough to overcome neural compensation and produce observable behavioral deficits. This suggests that perhaps single injection of βA may be responsible for the inconsistent behavioral impairments reported in the literature. A single injection may not produce lesions extensive enough to produce observable behavioral deficits.

Recently, attempts have been made to produce more extensive βA lesions by administering multiple injections or chronically infusing βA over a prolonged period, in order to attain behavioral spatial learning deficits. Nitta, Itoh, Hasegawa, and Nabeshima (1994), found that infusion of βA(1-40) into a cerebral ventricle of a rat over 14 days using a mini-osmotic pump led to a subsequent behavioral spatial learning impairment on a water maze task. In another experiment, the behavioral effects of 2 treatments of βA(25-35) injections bilaterally into the hippocampal areas CA1-3 were evaluated on a water maze task (Chen, Wright, & Barnes, 1994). It was reported that rats receiving multiple injections of βA(25-35) displayed a spatial learning impairment, exhibiting significantly longer escape latencies and pathlengths to the platform than controls (Chen et al.). These studies suggest that multiple injections or chronic administration of βA peptides may produce not only the specific neuropathological effects associated with Alzheimer's disease, but also the resulting behavioral learning deficits.

These techniques can be employed in order to determine the role of βA toxicity on behavior following multiple injections or chronic administration into various regions of the brain that are known to degenerate in AD patients. In review, it has been previously reported that injections of βA(25-35)
Effects of Multiple Injections of βA (25-35) into the medial septal area (MSA) of rats produces a hypofunction of the cholinergic system with a decrease in ACh release in the hippocampus (Abe et al., 1994). Doman, Tinkler, Litwiller, Fan, and Hanin (1996) used the technique of multiple injections to further investigate the neural pathway from the MSA to the hippocampus, referred to as the septo-hippocampal pathway. Injections of 5nm of βA(25-35) were given into three different depths of the MSA in male rats and spatial learning ability was then assessed using the Morris water maze and radial arm maze (Doman et al.). They report a mild behavioral memory impairment in these animals compared to controls receiving saline injections when they were tested on the radial-arm maze task (Doman et al.).

It was the aim of this study to obtain additional data to test the hypothesis that 5nm injections of βA(25-35) into three different depths of the MSA leads to degeneration of the septo-hippocampal pathway resulting in spatial learning and memory impairments. Establishing a causal link between βA, degeneration of specific areas such as the septo-hippocampal pathway, and memory impairments, would aid in the development of a useful animal model of the pathology of Alzheimer’s disease and perhaps in the elucidation of the role βA plays in the etiology of Alzheimer’s disease.

Method

Subjects

Eleven 10 month old aged male Long-Evans rats from the Illinois Wesleyan University animal colony were used. These rats were matched to the age used in the previous investigation by Doman et al (1996). The rats were housed individually in a temperature controlled environment at 20 degrees Celsius on a 12:12 light/dark cycle (lights on at 6:00 and off at 18:00). Food and water were available ad lib throughout the experiment except during radial-arm maze testing when rats were maintained at 85% of their previous body weight.
Apparatus

Behavioral testing of spatial learning ability was assessed using the Morris water maze (MWM) and the radial-arm maze (RAM). These spatial learning tasks require that rats learn and perform tasks requiring memory of the locations determined by spatial cues, memory of the rules of the maze, and memory of what they have already done and locations they have already been. The MWM consists of a circular fiberglass pool (Advanced Composite Technologies) measuring 183 cm in diameter which was filled with water (26°C) and rendered opaque by the addition of a non-toxic white tempera paint. The maze is separated into four quadrants labeled NE, SE, SW, NW. The RAM consists of a central chamber from which eight arms, with removable plexiglass barriers, protrude at 45° angles from the adjacent arms on either side. During all testing the rat was introduced into the central chamber. Both mazes were located in individual observation rooms which are filled with salient visual cues which remained unchanged throughout the course of the experiment. All data were collected with the aid of a computerized tracking system.

Procedures

Surgeries For all surgeries, animals were anaesthetized with a 100mg/kg intra-peritoneal (IP) injection of a Ketamine/Xylazine cocktail, comprised of 10ml of 100mg/ml Ketamine and 1.5ml of 100mg/ml Xylazine. Animals received stereotaxically implanted 22-gauge stainless steel guide cannulas with inner stylets (to prevent occlusion). Cannulas were directed above the medial septal area (from bregma, AP=+0.8 mm, ML=0.0 mm, DV=-5.2 mm) using the atlas of Paxinos and Watson (1986) as a guide. The cannulas were secured with cranioplastic cement.

Intracerebral Injections Each rat received 3 injections of either βA(25-35) (n=5) or vehicle (n=6) into 3 different depths of the MSA. The vehicle for the βA(25-35) injections was a solution of 35% Acetonitrile and 0.1% Trifluoroacetic acid in distilled water. Prior to injection all rats were
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anesthetized with a 100mg/kg Ketamine/Xylazine cocktail. Thirty-gauge injector cannulas were
inserted into the chronically implanted guide cannula that protruded into the MSA. The injector
cannulas were used to infuse 1μl of βA(25-35) at a dose of 5nm/μl or vehicle at a depth of DV=−6.2
mm. All injections were infused using a Stoelting micro infusion pump over the course of a half-hour
and the injector was removed five minutes after the injection was complete in order to limit diffusion
up the needle track. The animals were kept warm with the aid of a heating pad until they become self-
ambulating. Seven days following the first infusion, all rats were again anaesthetized and received a
second infusion of βA(25-35) or vehicle which was administered identical to the first infusion only
deeper into the MSA at a depth of DV=−7.2. Seven days after the second infusion, all rats were
anaesthetized again and received a third infusion of βA(25-35) or vehicle which was administered
identically to the first infusion only deeper into the MSA at a depth of DV=−8.2. Following the third
infusion all cannulas were immediately plugged with bone wax.

Morris Water Maze Testing Three days following their last infusion, all rats were tested on the
MWM. Rats were tested four times per testing session. For each trial, the animal was placed at one of
the four start locations (in the middle of the NE, SE, SW, or NW quadrant) such that each start location
was used once each testing session. A random sequence of start locations was used on each day of
testing. Each animal was allowed 90 seconds to find a submerged platform which was located 2 cm
below water level. If the animal failed to find the platform during the 90 second interval, it was gently
guided to it and left on the platform for 10 seconds. After the animal had remained on the platform for
10 seconds it was removed from the pool and ten seconds were allowed to lapse before the next trial
began. After the four trials were completed, the rat was removed from the pool, dried and returned to
its cage. Escape latency (the amount of time required to locate the platform in seconds), pathlength (in
pixels), and swimspeed (in pixels/second) were recorded. Differences were expected between groups
Effects of Multiple Injections of βA (25-35) on escape latency and pathlength, but not on swimspeed.

On the first day of MWM testing, all rats were assessed using a non-cued task where the platform was not visible. The submerged platform was located and remained in the NW quadrant for all non-cued testing trials. Non-cued memory testing was performed once each day over the course of five consecutive days.

Immediately following the completion of non-cued testing trials the task was changed to a reversal task. In this task the platform was moved from the NW quadrant to the SE quadrant in order to assess the ability of rats injected with βA(25-35) and vehicle to adapt to the change in conditions and learn the new location of the platform. This type of response perseveration characterized by a difficulty in changing and adapting strategies to a new task has been noted in patients afflicted with Alzheimer's disease. The presence of perseverative behavior was assessed in this task, but not expected due to perseverative behavior being considered a result of frontal lobe damage. Reversal task testing was performed for two consecutive days immediately following the last day of noncued testing in the MWM.

Immediately following the completion of reversal testing trials the task was changed to a pharmacological challenge task. In this task, the effects of IP injections of the cholinergic muscarinic receptor blocker scopolamine was assessed by performance of a reversal task. This was assessed in order to determine if cholinergic dysfunction is present but not severe enough to result in behavioral impairment. If injections of βA(25-35) compromised the cholinergic system it would be expected that injections of scopolamine would further reduce cholinergic activity by blocking the receptors on the remaining cholinergic neurons, facilitating prior cholinergic deficits. Scopolamine injections would act to potentiate the effects of a prior cholinergic depletion in animals that had received βA(25-35) where animals with a normal cholinergic system would be able to compensate. This would lead to
more drastic behavioral impairment in animals who had a compensated cholinergic system as a result of βA(25-35) injections.

Four days of a pharmacological challenge task was conducted immediately following the last day of reversal testing. All rats received IP injections of Scopolamine hydrobromide in physiological saline at a dose of .3 mg/kg on days 1 and 3 while no additional treatment was given on days 2 or 4 in order to assess recovery from the drug. All rats were tested 15 minutes after receiving injections. Before the scopolamine testing trials began the platform was moved from the SE quadrant to the SW quadrant. Scopolamine has a short half-life and no carryover effects of the drug are expected to interfere with testing on the days when the drug is not administered.

Radial-Arm Maze Testing Following MWM testing, spatial learning ability was assessed using the radial-arm maze (RAM). The RAM not only tests memory of locations based on spatial cues, but also tests proactive and retroactive memory errors in a delayed non-matching to sample task. Following MWM testing, all rats were food deprived and maintained at 85% their previous body weight. Three days following the completion of MWM testing and the initiation of food deprivation they were exposed to a 3-day adaptation phase using the RAM. During this phase, reinforcers (Cheerios) were liberally scattered on the RAM. The animals were placed on the center of the maze and allowed to explore for 5 minutes. Following the adaptation trial on the third day the last phase of adaptation was completed, all animals were once again introduced to the maze that same day for five minutes with only the ends of each arm baited as they were in further testing.

Following the adaptation phase, each animal was tested once each day for nine days. Animals were introduced to the RAM twice during each testing session. In the first trial, or pre-delay trial, four arms were blocked off and the remaining four arms were baited with the reinforcer. Each rat was placed in the center of the RAM and allowed five minutes to visit the four open arms. All rats were re-
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introduced to the RAM after an hour delay, all eight arms were open, and the four arms that were previously blocked were baited in this delay condition. The configuration of the blocked arms were never repeated and were changed on a daily basis. This type of task is often referred to as a "delayed-non-matching to sample task" or DNMTS. During the delay trials the following behavioral parameters were recorded: 1) retroactive memory errors, entries into previously baited arms, and 2) proactive memory errors, number of reentries into arms previously visited in this trial. These types of memory errors display more immediate and post-delay memory loss (proactive and retroactive, respectively) that may parallel short and longer term memory deficits noted in patients with Alzheimer's disease (Berg & Morris, 1994).

Histological Verification One week following the last day of behavioral testing, all animals were sacrificed, perfused, and the brains removed. The brain of one animal from each group was sectioned and stained with Cresyl Violet in order to verify the location of the cannula placement. Further histological analysis will examine the medial septal area of all remaining animals receiving injections of βA(25-35) and a control animal from each group, sectioning and staining with a Congo red histological stain in order to assess the level of aggregated βA plaques in the MSA.

Results

Morris Water maze

Rats receiving injections of βA(25-35) performed no different than rats receiving injections of vehicle on all MWM tasks. For all tests the Bonferroni index was used, utilizing a more conservative alpha level of .001 in order to take into account the large number of unweighted means analyses of variance which were performed. In the non-cued task no significant effects of injection were found on escape latency \([F (1, 54) = .0026, p > .9]\)(see fig. 1), pathlength \([F (1, 54) = .11 p > .7]\)(see fig. 2), or swimspeed \([F(1, 54) = .15, p > .7]\)(see fig. 3). These results along with a non-significant day-by-
Effects of Multiple Injections of βA (25-35)

Injection condition interactions for escape latency [F (4, 54) = 1.33, p > .2], pathlength [F(4, 54) = .92, p > .4], and swimspeed [F(4, 54) = 1.38, p > .2] indicate that multiple injections of βA(25-35) into the MSA had no significant effects on water-maze performance in the non-cued trials when compared to controls. A main effect of days was noted on escape latency [F (4, 54) = 14.13, p < .0001], pathlength [F (4, 54) = 7.67, p < .0001], and swimspeed [F(4, 54) = 16.88, p < .0001] revealing that both groups learned the task and significantly improved over days.

Similarly, in the reversal trials, no significant effects of injection condition were found on escape latency [F (1, 21) = .92, p < .3] (see fig. 4), pathlength [F (1, 21) = .39, p < .5] (see fig. 5), or swimspeed [F(1, 21) = 1.00, p > .3] (see fig. 6). A marginal effect (but not significant at an alpha level of .001) of injection condition was noted on time spent in quadrant the platform was previously located with control animals spending more time in the quadrant than animals injected with βA(25-35) [F(1, 21) = 6.7, p < .03] (see fig. 7). No significant main effect of days was noted for all parameters signifying that both groups failed to significantly improve on the reversal task from the first day to the second day of trials. No day-by-injection condition interactions were found for escape latency [F (1, 21) = .30, p > .5], pathlength [F (1, 21) = 0.45, p > .5], swimspeed [F (1, 21) = 0.08, p > .7], or time spent in previous quadrant [F(1, 21) = .31, p > .5]. Collectively, this data indicates that multiple injections of βA(25-35) into the MSA failed to have an appreciable effect on water maze performance in the reversal trials when compared to controls.

On days where the cholinergic antagonist scopolamine was administered in the pharmacological challenge task, no significant main-effects were noted between animals receiving injections of βA(25-35) and controls. No significant effects of injection condition were found on escape latency [F (1, 21) = .11, p > .7] (see fig. 8), pathlength [F (1, 21) = .17, p > .6] (see fig. 9), or swimspeed [F(1, 21) = .93, p > .3] (see fig. 10). While a mild effect of days was observed on escape
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latency \[ F(1, 21) = 11.69, p < .008 \] (see fig. 8) and pathlength \[ F(1, 21) = .012, p > .02 \] (see fig. 9), denoting improvement over the course of the task, this was not sufficient to display a significant effect of days at a conservative alpha level of .001. As in both previous water maze tasks, no significant day-by-injection condition interactions were observed for escape latency \[ F(1, 21) = .075, p > .7 \], pathlength \[ F(1, 21) = 0.85, p > .8 \], swimspeed \[ F(1, 21) = .39, p > .5 \]. In summary, no significant effects of injections of βA(25-35) into the MSA were observed on spatial learning as assessed on the Morris water maze.

Radial-Arm maze

The data was averaged into blocks of three days for statistical analyses. Data analysis began on day six of the RAM testing (by day six most animals had begun to consistently perform the task). For all tests a more conservative alpha level of .001, in accordance with the Bonferroni index, was used in order to take into account the large number of ANOVAs that were performed within this study. An unweighted means analysis of variance (mixed design) with blocks as the repeated measure, and injection condition as the between measure, on retroactive memory errors revealed no significant main effect of injection condition \[ F(1, 77) = 2.86, p > .1 \] (see fig. 11) or block-by-injection condition interaction \[ F(1, 77) = .97, p > .3 \]. Similar statistical analysis on proactive memory errors failed to find a significant effect of injection condition \[ F(1, 77) = .88, p > .3 \] (see fig. 12) or block-by-injection condition interaction \[ F(1, 77) = 1.6, p > .2 \]. A moderate effect of blocks was displayed for both retroactive memory errors \[ F(2, 77) = 5.33, p < .008 \] and proactive memory errors \[ F(2, 77) = 4.24, p < .02 \] displaying improvement of all animals over blocks, but both measures failed to be statistically significant at a more conservative alpha level of .001. These results reveal that multiple injections of βA(25-35) into the medial septal area had no significant effects on the radial-arm maze task when compared to controls.
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Neurochemical Analysis and Histochemical Verification

Analysis of brain sections stained with Cresyl Violet revealed that the location of the cannula placements examined were directed at the Medial Septal area as intended in the surgical procedure. Further histological analysis of the brains of these animals will be done to determine the effects of injection condition on the brain areas of interest.

Discussion

The present study displays that three injections of βA(25-35) into three different depths of the medial septal area of the male rat fails to induce behavioral spatial learning deficits as assessed on the Morris water maze and radial-arm maze. In contrast to the results of this study, many recent studies on the behavioral effects of different βA fragments following multiple injections (Chen et al., 1996; Dornan et al., 1996) or chronic administration (Nitta et al., 1994) of these peptides into various regions of the brain associated with learning and memory function have found spatial learning impairments. A study by Dornan et al. (1996) assessing the effects of multiple injections of βA(25-35) into the MSA employed identical surgical, injection, and behavioral testing procedures and found a marginal effect on spatial learning accompanied by a disruption of the cholinergic septo-hippocampal pathway which was noted 60 days following the last injection. The present study failed to replicate these findings and provides contrary evidence as to the behavioral effects of multiple injections of βA(25-35) into the MSA.

Curiously, not only did this study fail to find any significant effects of injection βA(25-35) on spatial learning, but the only effect on a trend towards significance was on time spent in the previous quadrant in the reversal MWM task. In this task, control animals spent more time in the quadrant the platform was previously located in than did animals receiving injections of βA(25-35). This displays a greater difficulty of control animals to adapt their search strategy. This trend was opposite of what
was expected given previous evidence. This effect could be due to a stronger memory of the previous location in control animals in comparison to those injected with βA(25-35). This is supported by similar escape latencies and pathlengths displayed in βA(25-35) animals on the last day of non-cued trials and in the reversal trials while the escape latencies and pathlengths of control animals dramatically increased the first day following the change in task. This difficulty of the control animals may have been due to a stronger knowledge of the previous non-cued task while weaker memory of the task in βA(25-35) animals may have caused less of a problem for these animals in adapting to a new task. Perseveration in βA(25-35) animals, denoted by a more time spent in quadrant the platform was previously located in, would not be expected given no frontal lobe damage, which is believed to be responsible for perseverative behavior, should be present in these animals. The combined results of this study, in contrast to previous findings, do not support that multiple injections of βA(25-35) into the MSA produce spatial learning deficits in the male rat.

One possible explanation of the lack of spatial learning deficits following these injections of βA(25-35) is that no cholinergic depletion was present and no degeneration of the septo-hippocampal pathway occurred as a result of these injections. Further histological examinations will aid in revealing if this is indeed the explanation of the observed results of this study, or whether other implications are warranted from these results. In future study, the histology of these animals will be examined in order to determine if aggregated amyloid plaques or cavitation of brain tissue is present. Cavitation of brain tissue is a primary indicator of cell death and observed cavitation would support the presence of a neurochemical depletion within the brain areas as a result of βA injections. The presence or absence of neuritic plaques, comprised of aggregated βA(25-35), in the MSA which are thought to be responsible for disruption of the septo-hippocampal pathway may also aid in determining whether a neurochemical depletion may be present. An additional experiment, which is currently in
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progress, again replicated these surgical procedures of this study will assess the neurochemical effects of three identical injections of βA(25-35) into the MSA at a shorter interval of three weeks following the final injection. These future studies will aid in establishing whether this methodology produces consistent and reliable cholinergic depletions as that previously reported (Dornan et al., 1996).

One possible explanation for the lack of any marked effect on spatial learning, even despite reduction of cholinergic input to the hippocampus could be the result of postsynaptic up-regulation of hippocampal muscarinic receptors. This hypothesis was tested using a pharmacological challenge task. No differences, however, were observed on escape latencies, pathlengths or swimspeeds between the two groups following I.P. injections of the muscarinic receptor blocker scopolamine. This supports the hypothesis that a profound cholinergic depletion was not present in the animals used in this study.

The greatest limitation of this study is that it does not directly observe neurochemical levels in the MSA. Without this data, it will remain unknown if the lack of behavioral impairment was the result of a failure to produce cholinergic degeneration of the septo-hippocampal pathway or if the previously reported marginal impairments on the RAM following identical procedures (Dornan et al., 1996) was due to random differences in the animals tested. Histological data over the course of the experiment would also aid in developing a fuller understanding of the role of βA(25-35) in this experiment. There are no data to suggest whether previously reported disruptive effects of the βA(25-35) injections were progressive over the course of the experiment, whether compensation and recovery occurs, or if the effects will remain fairly constant over the course of the study. This data would not only aid in the elucidation of the role of βA(25-35), but would also clarifying whether the βA administration methodology used in this experiment produces a cholinergic depletion. It is for this reason that a study is in progress in order to determine the histological and neurochemical effects of the identical surgical procedures after a three week period of time. One additional limitation of the
study is the lack of a control group not receiving any surgical procedures in order to assess performance of a normal rat on these spatial learning tasks and allow for comparison.

Despite the lack of significant results in this study, it has raised questions concerning the conclusions of Dornan et al. (1996) and the behavioral effects of βA(25-35) following multiple injections into the MSA. These findings will lead to further experimentation in order to resolve the conflicting evidence which has risen from this study. This further experimentation will aid in the development of an animal model of Alzheimer's disease and perhaps lead to the elucidation of the exact role βA plays in the etiology of Alzheimer's disease.
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References


Figure Captions

Figure 1. Mean escape latencies to a hidden platform in rats following multiple injections of βA (25-35) or vehicle into the MSA were measured on a non-cued Morris water maze task. Each rat received four tests per day for 5 consecutive days. Vertical lines represent +S.E.M. See text for details.

Figure 2. Mean pathlengths to find the hidden platform in rats following multiple injections of βA (25-35) or vehicle into the MSA were measured on a non-cued Morris water maze task. Each rat received four tests per day for 5 consecutive days. Vertical lines represent +S.E.M. See text for details.

Figure 3. Mean swimspeeds onto a hidden platform in rats following multiple injections of βA (25-35) or vehicle into the MSA were measured on a non-cued Morris water maze task. Each rat received four tests per day for 5 consecutive days. Vertical lines represent +S.E.M. See text for details.

Figure 4. Mean escape latencies to a hidden platform in rats following multiple injections of βA (25-35) or vehicle into the MSA were measured on a reversal Morris water maze task. Each rat received four tests per day for 2 consecutive days. Vertical lines represent +S.E.M. See text for details.

Figure 5. Mean pathlengths to find the hidden platform in rats following multiple injections of βA (25-35) or vehicle into the MSA were measured on a reversal Morris water maze task. Each rat received four tests per day for 2 consecutive days. Vertical lines represent +S.E.M. See text for details.

Figure 6. Mean swimspeeds onto a hidden platform in rats following multiple injections of βA (25-35) or vehicle into the MSA were measured on a reversal Morris water maze task. Each rat received four tests per day for 2 consecutive days. Vertical lines represent +S.E.M. See text for details.
Effects of Multiple Injections of βA (25-35) 22

Figure 7. Mean times spent in the quadrant the hidden platform was previously located following multiple injections of βA (25-35) or vehicle into the MSA of rats were measured on a reversal Morris water maze task. Each rat received four tests per day for 2 consecutive days. Vertical lines represent +s.e.m. See text for details.

Figure 8. Mean escape latencies to a hidden platform following I.P. injections of scopolamine as measured on a reversal Morris water maze task. Each rat received four tests per day on 2 days with a day of non-drug testing following each day of drug testing. Vertical lines represent +s.e.m. See text for details.

Figure 9. Mean pathlengths to find the hidden platform following I.P. injections of scopolamine as measured on a reversal Morris water maze task. Each rat received four tests per day on 2 days with a day of non-drug testing following each day of drug testing. Vertical lines represent +s.e.m. See text for details.

Figure 10. Mean swimspeeds onto a hidden platform following I.P. injections of scopolamine as measured on a reversal Morris water maze task. Each rat received four tests per day on 2 days with a day of non-drug testing following each day of drug testing. Vertical lines represent +s.e.m. See text for details.

Figure 11. Effects of multiple injections of βA (25-35) into the MSA on the mean number of retroactive memory errors on a standard RAM task with a 1 hour delay. Retroactive memory errors are defined as entries into unbaited arms that were baited and visited during the pre-delay session of the trial. Vertical lines represent +s.e.m. See text for details.

Figure 12. Effects of multiple injections of βA (25-35) into the MSA on the mean number of proactive memory errors on a standard RAM task with a 1 hour delay. Proactive memory errors are defined as re-entries into any arm during the post delay session of the trial. Vertical lines represent
+s.e.m. See text for details.
NON-CUED TASK
Days Vs. Escape Latency

- Controls
- Beta-Amyloid (25-35)

Days

Escape Latency (Seconds)

Figure 1
Effects of Multiple Injections of βA (25-35)

NON-CUED TASK
Days Vs. Pathlength

- Controls
- Beta-Amyloid (25-35)

Mean Pathlength (Pixels)

Days

Figure 2
NON-CUED TASK
Days Vs. Swimspeed

Figure 3
REVERSAL TASK
Days Vs. Escape Latency

- Controls
- Beta-Amyloid (25-35)

Figure 4
REVERSAL TASK
Days Vs. Pathlength

- Controls
- Beta-Amyloid (25-35)

Figure 5
Effects of Multiple Injections of βA (25-35)

REVERSAL TASK
Days Vs. Swimspeed

- Controls
- Beta-Amyloid (25-35)

Figure 6
Effects of Multiple Injections of \( \beta A (25-35) \)

**REVERSAL TASK**

Days vs. Time Spent in Previous Quadrant

- **Controls**
- **Beta-Amyloid (25-35)**

![Figure 7](image)

*Figure 7*
PHARMACOLOGICAL CHALLENGE
Days Vs. Escape Latency

- Controls
- Beta-Amyloid (25-35)

Escape Latency (Seconds)

Days Received Scopolamine

Figure 8
PHARMACALOGICAL CHALLENGE
Days Vs. Pathlength

Figure 9
Effects of Multiple Injections of βA (25-35)

**PHARMACOLOGICAL CHALLENGE**

Days Vs. Swimspeed

![Graph showing mean swimspeed (pixels/second) over days for controls and Beta-Amyloid (25-35).](image)

Figure 10
Radial-Arm Maze Task
Blocks Vs. Mean Retroactive Memory Errors

- Controls
- Beta-Amyloid (25-35)

Figure 11
Effects of Multiple Injections of βA (25-35) 35

Radial Arm Maze Task
Blocks vs. Mean Proactive Memory Errors

- Controls
- Beta-Amyloid (25-35)

Figure 12