1993

An Animal Model of Alzheimer's Disease: Behavioral and Histological Assessment Following Bilateral Intrahippocampal Injections of B-Amyloid (25-35)

David E. Kang '93
Illinois Wesleyan University

An Animal Model of Alzheimer's Disease: Behavioral and Histological Assessment Following Bilateral Intrahippocampal Injections of ß-Amyloid (25-35)

David E. Kang

Illinois Wesleyan University

1993

Running Head: ANIMAL MODEL OF AD

Acknowledgements

I would like to first express my gratitude to Dr. Wayne Dornan for his felicitous direction, confident support, and exemplary leadership as a teacher, researcher, and supervisor throughout the progress of this study. I would also like to thank my sister, Esther Kang, and Alex McCampbell who played integral role in behavioral testing and immunocytochemistry, without whose help this research would not have been possible. I would like to thank Jason Bubberel for his knowledgeable assistance in immunocytochemistry and Harinie Wijiwiera for timely substitute help in behavioral testing.

Abstract

Pathologically, Alzheimer's disease (AD) is characterized by deposits of neuritic plaques (NP) and neurofibrillary tangles (NFT) typically found in the cerebral cortex, hippocampus, and basal forebrain. Increasing evidence suggests that the major constituent of NP, a ß-amyloid protein (ßAP) composed of 39-42 amino acids, possesses neurotoxic properties. It has been reputed that the neurotoxic properties of ßAP(1-40) may be dependent on the aggregational state of the peptide. Recent studies have demonstrated *in vitro* that a fragment of the ß-amyloid protein, ßAP(25-35), disrupts intracellular calcium homeostasis, decreases neuronal survival, and potentiates the toxicity of excitatory amino acids (EAA). While some evidence supports the direct *in vivo* toxicity of ßAP, the extent of neuronal damage has not been compared with standard lesions made by EAAs. Moreover, currently the effects of intracerebral injections of ßAP(25-35) on learning and memory in the rat is unknown. Therefore, in this study a comparative behavioral and histological assessment was conducted following bilateral intrahippocampal injections of ßAP(25-35), ibotenic acid (IBO), ßAP(25-35)+IBO, and incubated ßAP(25-35) (1 week at 37 C). A radial arm maze and Morris water maze were utilized for comparative learning

and memory assessment. Preliminary results indicate that there is a clear disruption of learning performance in animals co-injected with low doses of ßAP(25-35)(4nmol/ul) + IBO (1ug/ul) while identical doses injected separately had no effect. The deficits in learning observed following injections of ßAP(25-35)+IBO and the high does of IBO was not, however, due to disruptions in motor behavior as there was no difference found between groups on a treadmill test in those that ran the maze and those that did not. In support of the behavioral results, preliminary histological analyses revealed cytotoxic effects in the hippocampus following injections of ßAP(25-35) + IBO or a high does of IBO. This study suggests that the injection of ßAP(25-35) into the hippocampus promotes the vulnerability of neurons to excitotoxic damage *in vivo* and disrupts learning/memory in rats.

The number of people afflicted by neurodegenerative disorders has been increasing. Of particular interest is Alzheimer's disease (AD), which is characterized by progressive decay in cognitive abilities (e.g. memory, reasoning capability) associated with neuronal degeneration and synapse loss. Alzheimer's disease is the 4th leading cause of adult death in the U.S. after heart disease, cancer, and stroke, claiming more than 100,000 live per year. If no cure is found, the number of individuals affected by AD will double by the year 2000 and quintuple by the year 2040 [7]. Currently, neither the etiology nor the pathogenesis of AD is clearly understood. Therefore, it is evident that Alzheimer's disease will become the epidemic of the future if no effective treatment is found.

Neurohistological and neuroanatomical analyses have revealed that the pathological hallmark of AD is the accumulation of neuritic plaques (NP) and neurofibrillary tangles (NFT), typically found in the cerebral cortex, hippocampus, and basal forebrain [19]. These lesions have been found to accompany substantial synapse loss and neuronal degeneration [20]. However, whether neurodegeneration is caused by plaque accumulation or plaque formation is somehow

a result of cell death is somewhat controversial.

Neuritic plaques are composed of a core component, the ß-amyloid protein (ßAP) which consists of 39-42 amino acids [9] and is enzymatically cleaved from a larger amyloid precursor protein (APP) by an unknown pathogenic mechanism and is deposited in the extracellular matrix, progressively developing from a diffuse, soluble state to a neuritic, insoluble form [13]. In contrast, neurofibrillary tangles are filamentous intracellular deposits that are found in cell bodies and neurites of neurons that are marked for eventual degeneration [6]. NFT are relatively insoluble and remain when the death of the tangle-bearing neuron is complete, so called "ghost tangles". Paired helical filaments (PHF) are the major structural components of NFT and tau, a microtubule associated protein, is an integral constituent of PHFs [3]. Presumably, recent evidence suggests that a hyperphosphorylation event occurs to the tau protein in degenerating neurons [12]; therefore, antibodies which recognize the phosphorylated tau (e.g. Alz-50) have been widely utilized to immunostain tangle-bearing neurons and normal nerve cells that are at risk of developing neurofibrillary pathology [6].

Substantial data suggest that the accumulation of ßAP

occurs prior to tangle formation; therefore, mature ßAP may be the cause of NFT induction, and thus neuronal degeneration [14]. Whether this event is the pathogenic mechanism of cell death in AD is uncertain. Nevertheless, increasing evidence suggests that ß-amyloid protein possesses neurotoxic properties. In 1991, Yankner and Kowall reported that ßAP (1-40), when injected into the hippocampus of rats, causes neurodegeneration and a marked induction of Alz-50 immunoreactivity [11]. While several laboratories were able to observe the *in vivo* neurotoxicity of ßAP along with corresponding antigenic changes, however, others were unable to replicate Yanker's initial findings. For instance, Podlisny et al. injected synthetic ßAP (1-40) in monkey cerebral cortex but found no cellular changes resembling AD pathology [17].

One possible explanation for these conflicting reports may be related to the self-aggregational properties of ßAP(1-40) which nos not controlled for in earlier studies. Pike et al. reported that when freshly solubilized (new) ßAP(1-42) is applied, there is no difference in neuronal survival of hippocampal cultures when compared to saline controls while identical doses of incubated ßAP(1-42)(7 days at 37 C), however, dramatically reduced neuronal survival [15]. A later

study by Pike and colleagues confirmed their previous results and showed that the incubation of βAP(1-42) under physiological conditions adopts an aggregated, insoluble form and exerts neurotoxicity in cultured neurons. Following incubation, ß-pleated sheet-like aggregates were observed under a light microscope [16].

Until very recently, the molecular mechanisms of βAP(25-35)-induced neurotoxicity has not been investigated. Yankner et al. recently reported that βAP(1-40) is neurotoxic to differentiated hippocampal cultures, and that the neurotoxic activity resides within the amino acids 25-35 of the full peptide [21]. Furthermore, Joseph and Han reported that this putative neurotoxic fragment, βAP(25-35), causes a specific and dose-dependent increase in intracellular calcium by an influx of extracellular calcium in PC12 cultured neurons [8]. Arispe and colleagues later showed that βAP(1-40) increases intracellular calcium by spanning the bilipid membrane and forming calcium channel-like ionophores. It has been suggested that the formation of calcium channels is possible only when the βAP polymerizes into stable aggregates (dimer, trimers, tetramers, etc.) due to the relative short length of the peptide [1]. In addition, Hu and El-Fakahany showed that βAP(25-35) stimulates the formation of cGMP via a calcium

dependent increase in nitric oxide synthase (NOS) in cultured PC12 neurons [unpublished manuscript]. Several years earlier, Koh et al. (1990) had reported that ßAP potentiated the toxicity of excitatory amino acids (e.g. ibotenic & kainic acid) in cultured cortical neurons [10]. Collectively, these results suggest that a mechanism of action related to the pathology of Alzheimer's disease may be via a synergistic action of ßAP with an excitatory amino acid (e.g. glutamate), which leads to a disruption of cellular calcium homeostasis. This process may be further exacerbated by the aggregational properties of ßAP.

One of the structures that has been found to be selectively targeted for degeneration in brains of AD patients is the hippocampus [19]. In an effort to establish an animal model of Alzheimer's disease, the present study compared the effects of excitotoxic lesions of the hippocampus with intrahippocampal injections of different doses of ßAP(25-35) on learning/memory in the rat. Previous studies have shown that excitotoxic lesions using different glutamate analogs produce dramatic effects on learning when injected into the hippocampus. In order to examine the specificity of the lesions, histological as well as immunocytochemical procedures were employed. The behavioral paradigms used in the present

study were the 8-arm radial arm maze, Morris Water Maze, and a treadmill test for motor activity. It was hypothesized that there would be a dose and aggregation dependent neurotoxic action of ßAP(25-35), and that ßAP(25-35) will potentiate the toxicity of ibotenic acid. The learning and memory performance of the groups should thus mirror the extent of lesions made by the injections.

Method

Animals and Surgery

In a total of 39 Long-Evans rat, stereotaxic surgery was performed. Three of these rats died as a result of the surgery (ßAP(high), ßAP(low), and ßAP(high-incubated)), 2 died during RAM testing (2 salines) and 1 died immediately upon the completion of the RAM testing (ßAP(low)). They were individually housed and maintained in a controlled light/dark cycle environment (lights on 6:00 and off at 21:00). ß-amyloid(25-35) was purchased from Bachem Inc. (Torrence, CA) and ibotenic acid from Sigma Chemicals (St. Louis, MO). Eight to 10 days prior to testing, rats were anesthetized with sodium pentobarbital (50mg/kg) and placed in a sterotaxic surgery apparatus in the flat-skull position. A burr hole was

made through the skull and a 2-ul Hamilton syringe was lower down to the desired coordinates (AP=-3.6, ML=±2.3, DV=-3.6) over a period of 3 minutes. ßAP(25-35) and ibotenic acid (IBO) were dissolved in 1ul of vehicle (phosphate buffered saline), and all groups received bilateral injections into the hippocampus over a period of 4 minutes . The groups were **group 1)** ßAP(25-35) low (n=3), 4 nmol; **group 2)** ßAP(25-35) high (n=4), 8nmol; **group 3)** IBO low (n=4), 1ug; **group 4)** IBO high (n=4), 2.8ug; **group 5)** ßAP(25-35) low + IBO low (n=6); **group 6)** ßAP(25-35) high (n=5), incubated at 37 C for 7 days; **group 7)** phosphate buffered saline. In order to prevent back flow and minimize tissue damage, the syringe was left in place for 5 minutes and removed over a duration of 3 minutes. Following surgery, animals were given 10-12 days for recovery.

Radial Arm Maze

Beginning the first day of habituation, rats were regularly handled to minimize stress and maintained at 80-90% of their *ad libitum* weight for the duration of radial arm maze and locomotor testing. The 8-arm radial arm maze (RAM) was made of plywood painted black and stood 30 inches high with a total diameter of 72 inches. Each arm extended a length of 30 inches from the perimeter of the center platform, which had a

diameter of 12 inches. Each subject was randomly assigned an initial maze orientation which remained fixed for the duration of the experiment. This was to control for any possible directional preference with respect to external cues. Prior to beginning RAM testing, rats were given 8 days (5 min/day) of habituation phase. During this period, the appetetive reinforcer (Froot Loops) were liberally scattered on the RAM; then they were individually placed onto the center platform and allowed to explore the RAM and acclimate to the new environment. Subjects were tested in a random alternating time schedule (alternating from a daily testing period of 9-10:30 AM or 12-1:30 PM or 2:30-4:00 PM). All behavioral testing was performed in a blind manner with 3 experimenters contributing to the effort daily. After each session, the maze was thoroughly wiped with a semi-wet towel to nullify any odor effects.

Initially 5 arms were randomly chosen and consistently baited every session. At the beginning of the testing period, each rat was placed at the center platform and permitted to choose among the arms until it either successfully completed the test (obtained all rewards) or until 10 minutes had elapsed. A white masking tape was taped around each arm 3 inches in front of the white bait cup, and an entry into an

arm was defined as two front paws beyond the tape. If no choices were made during the first 5 minutes of the session, animals were discontinued and taken off the maze.

Arm choices and time required to complete a session were recorded. A working memory correct error (WMCE) was defined as an entry into an arm from which the rat had already obtained food while reference memory error (RME) was determined as an entry into an arm that was never baited. In addition, a working memory incorrect error was defined as an entry in which the rat had already made a RME. Choice accuracy (ChA) was recorded as the number of correct choices made before making an error. In case rats did not enter arms, consistent entry (CE) of a subject was defined as at least 1 entry per session for at least 3 sessions per block (6 sessions). As a rough measure of the general activity of the rat, choice latency (ChL) was calculated as the session latency (SesL) divided by the total number of arms entered (Tch). In the fixed RAM design, working memory was comparable to short-term memory, because it was a measure of the subjects' ability to remember which arms were recently entered (seconds to at most 10 minutes). Reference memory, in contrast, was analogous to long term memory insofar as it was a measure of the rat's ability to remember which arms were

never baited over an extended period of time. RAM testing continued for 24 days (4 blocks).

Locomotor Behavior

Locomotor testing commenced 4 days following the completion of RAM. Rats were individually placed inside a treadmill from which they could not escape and were given 5 minutes to move freely. The number of revolutions made were recorded for the first 90 seconds and for the entire five minutes. Testing continued for 4 consecutive days.

Morris Water Maze

Upon the completion of locomotor testing, rats were put on a free feeding diet regime and given 7 days resting period prior to beginning the Morris Water Maze (MWM). The Water Maze resembled a circular pool which was 200 cm in diameter. Luke warm water (~30 C) was filled up to 15 inches high with the walls of the pool extending 6 inches above the surface of the water. The MWM was arbitrarily divided into 4 equal quadrants that were formed by imaginary lines which intersected at the center of the pool at right angles. An arbitrary direction (N, E, S, W) was given to where the imaginary lines met the wall of the maze. During the

habituation phase, animals were introduced to the water maze by individually placing them into the water from the North then the East, 90 seconds each. Habituation continued in this manner for 3 days. On the fourth day, testing began after a sufficient amount of powdered milk was added to the water as to hide the escape platform (2 cm below the water surface) which always resided in the middle of the SW quadrant. For eight days, rats were given two consecutive trials per day by placing them into the water (head facing the maze wall) from the North and East start positions (start positions alternating daily). For each trial, subjects were left to swim freely for 90 seconds or until the platform was found. If it was not found within 90 seconds, the rat was guided to the platform and allowed to rest there for 10 seconds. For each trial, escape latencies were timed and recorded.

Tissue preparation and Histologies

Eight to 14 days following the termination of behavioral testing, animals were anesthetized with an overdose of sodium pentobarbital and transcardially perfused through the ascending aorta of the heart with 300ml of PBS followed by 600ml of 4% paraformaldehyde. The brains were removed and post-fixed in 4% paraformaldehyde overnight and cryoprotected

in a 20% phosphate-buffered sucrose solution of 4% paraformaldehyde until the investigators were ready to process them for histological analysis. All brains were sectioned (20um) and stained for the analysis of hippocampal cytoarchitecture with cresyl violet, which stains nissel bodies that are found in all cell bodies. In addition, every other section of each brain was stained with congo red and were counterstained with hematoxylin in order to visualize any possible amyloid deposits and cell bodies surrounding them.

In 9 of the brains (2 from PBS group, 2 from ßAP+IBO group, and 1 each from other groups), GFAP and Alz-50 immunocytochemistry was also performed in addition to the cresyl violet and congo red stains. GFAP was utilized to visualize the extent of gliosis in hippocampal lesions and Alz-50 for immunolabeling the tau protein, which has been associated with neurodegeneration []. The sections (20um) were washed with PBS (pH 7.4) and blocked with 10% normal horse serum (NHS), and incubated for 36 hours with the mouse anti-GFAP primary antibody (1:200) or mouse monoclonal Alz-50 primary antibody (1:5) at 4 C. The sections were then washed and treated with a biotinylated anti-mouse secondary antibody (raised in a horse) followed by Avidin-Biotin Complex (ABC) and reacted with diaminobenzedine (DAB) with 0.001% hydrogen

peroxide. A control immunocytochemical procedure was conducted by omitting the primary antibody.

Statistical Analysis

The present study used a between-within subject mixed design (7 groups X 4 blocks for RAM and 7 groups X 8 days for MWM). Due to the difficulties in performing a repeated measures ANOVA, however, the radial arm maze and water maze data were tentatively analyzed using the oneway ANOVA. Selectively for each block (6 days/block) of the RAM and each session of the MWM, Oneway ANOVAs were performed using 'groups' as the independent variable in order to compare the means of 6 independent samples for each of the dependent measure (e.g. choice accuracy, escape latency, etc.). A post hoc Student-Newman-Keuls (SNK) procedure was used to reveals which group(s) were significantly different each other. For the locomotor data, a t-test was employed to compare the mean number of revolutions between the animals that consistently entered arms versus those that did not.

Results

Radial Arm Maze

The general trend showed that only IBO(H) and

ßAP(L)+IBO(L) groups were impaired in learning and memory performance on the RAM. During block 1 of testing, there were no significant differences between groups in any of the dependent measures, except the ßA+IBO group which made significantly less arm choices compared to the IBO(L) groups (SNK $p < 0.05$). None of the animals in the ßAP+IBO group met the criterion for consistently entering the arms in any of the blocks; therefore, dependent variables could not be measured and statistical analysis was omitted for the ßAP+IBO group except total number of choices (Tch) which revealed to be significantly lower than all other groups in all blocks ($p < 0.001$). By block 2, group effects showed that choice accuracy, total number of choices, working memory incorrect errors, and total errors were significantly different ($F(4,73)=3.951$ $p < 0.006$, $F(5,156)=9.023$ $p < 0.001$, $F(4,73)=3.987$ $p < 0.006$, $F(4,73)=2.5987$ $p < 0.05$, respectively). Post hoc SNK analyses revealed that the IBO(H) group was significantly impaired in choice accuracy and made significantly greater number of working memory incorrect errors and total errors (SNK $p < 0.05$) compared to the controls. While all other groups were not significantly different from the controls in block 3, groups effects were found in choice accuracy, working memory correct error, and

total error ($F(5,74)=3.5054$ $p < 0.001$, $F(5,74)=3.716$ $p < 0.005$, $F(5,74)=2.9693$ $p < 0.05$, respectively), which was mainly attributed to significant memory deficits manifested by the IBO(H) group in all of the above dependent measures (SNK $p < 0.05$). By block 4, group effects disappeared for all memory measures, but SNK post hoc analysis revealed that the IBO(H) group committed significantly more working memory incorrect errors than the ßAP(H) group. Statistical analysis demonstrated that ßAP(L) or ßAP(H) groups were not significantly different from the SALINE controls in any of the dependent measures in any of the blocks of the RAM.

Locomotor Behavior

No significant overall differences were found in the number of revolutions made between the animals that consistently entered arms versus those that did not during the first 90 seconds or the entire 5 minutes of locomotor testing ($F=1.47$ $p > 0.1$, $F=1.69$ $p > 0.05$, respectively. In addition, there were no significant differences between entering and not entering animals during each of the 4 days of locomotor testing in the first 90 seconds or 5 minutes ($p > 0.1$).

Morris Water Maze

No significant group effects were found until session 3 of testing ($F(5,20)=3.263$ $p < 0.05$), wherein the IBO(L) group demonstrated a significantly shorter escape latency than ßAP(L)+ßAP(L) (SNK $p < 0.05$), but not significantly different from any other group. All other escape latencies were not significantly different between groups for the remaining sessions ($p > 0.05$). While the control and IBO(L) groups showed consistently shorter escape latencies session after session, however, IBO(H) and ßAP(25+35)+IBO(L) groups demonstrated poor learning rates over the session. In fact, the ßAP(25-35)+IBO(H) group showed an extinction of learning during the final 2 sessions (7 and 8). While the general learning rates of the ßAP(25-35)(L) and ßAP(25-35)(H) did not appear to be impaired, these animals showed an irregular up and down "saw-shaped" learning curves.

Histologies

A thorough histological analysis of the hippocampal cytoarchitecture has not yet been performed. Preliminary analysis, however, suggests that there is a profound disruption of the hippocampus accompanied by reactive gliosis in 5 of the 6 animals injected with ßAP(25-35)(L)+IBO(L). Adjacent to sites of degeneration, Alz-50 immunoreactivity was

also markedly enhanced in the ßAP(25-35)(L)+IBO(L) group. In all groups, there was a disruption of the cerebral cortex immediately dorsal to the injection area due to the burr hole made by the drill. In 3 of the 5 animals injected with a high dose of ibotenic acid, profound lesions were evident in the hippocampus. In all other animals, there was no obvious damage of the hippocampal cytoarchitecture. A more thorough analysis must be conducted, however, to confirm intracerebral depositions of ßAP(25-35) with congo red and to quantitate the degree of each lesion.

In the 6 rats that were randomly selected from the groups (1 per group) for glial acidic fibrillary protein (GFAP) immunocytochemistry, glial cells were counted as a part of Esther Kang's project. These results showed that the experimental animals stained a greater number of GFAP immunopositive cells in the hippocampus than the saline control.

Discussion

The results of this study indicate that the newly solubilized ßAP(25-35) is not directly neurotoxic to hippocampal neurons *in vivo*. As others have suggested

[10,14], however, βAP(25-35) may be involved in promoting the vulnerability of otherwise healthy neurons to excitotoxic damage (e.g. ibotenic acid) presumably by disrupting the cells' ability to maintain calcium homeostasis. This is not surprising given that excitatory amino acids (e.g. glutamate) and their analogs (e.g. ibotenic acid) exert their toxicity by creating a rapid and sustained elevation in intracellular calcium, thereby interacting with βAP(25-35) at a cellular level [2]. While a low (4nmol/ul) or high dose (8nmol/ul) of βAP(25-35) or a low dose (1ug/ul) of ibotenic acid (IBO) injected separately had no appreciable effect on learning/memory nor produced significant lesions, co-injections of low doses of βAP(25-35)+IBO resulted in profound deficits in learning performance accompanied by severe necrosis of the hippocampus and reactive gliosis.

Not surprisingly, bilateral intrahippocampal injections of a high dose of IBO resulted in significant deficits in choice accuracy and working memory on the radial arm maze associated with marked disruption of hippocampal cytoarchitecture. These results are consistent with previous reports, suggesting that lesions of the hippocampus selectively impair working memory [4]. It is difficult to determine why none of the animals in the βAP(25-35)+IBO group

did not consistently enter arms in the RAM task, because their motor activity during a treadmill test was not significantly different from the controls, and no differences were found between the rats that ran the RAM and those that did not. However, it is noteworthy to mention that animals in the ßAP(25-35)+IBO group showed a diminished ability to acclimate to the stress-provoking radial arm maze environment; this was evident by freezing, urinating, and/or defacating on the center platform throughout the testing procedure. It is also possible that lesions due to co-injections of ßAP(25-35)+IBO are not completely selective to the injection ares and may indiscriminantly affect area associated with habituation and/or decision making.

While injections of ßAP(25-35)(L) or ßAP(25-35)(H) were not directly neurotoxic and did not impair performance on the RAM task, a "saw-shaped" learning curve was observed in the water maze task. While these rats did learn the maze, however, their learning was irregular and sporadic--markedly improving in 1 session and forgetting in the next. This phenomenon may be related to ßAP(25-35)'s ability to disrupt cellular calcium homeostasis since a fine regulation of intracellular calcium is crucial for hippocampal long-term potentiation (LTP), an electrophysiological phenomenon

intimately associated with learning/memory.

Collectively, the results of the present study suggest that ßAP(25-35) does exert action on neurons in the hippocampus, however, is not directly neurotoxic *in vivo*. Evidence suggests that ßAP(25-35) destabilizes intracellular regulation of calcium in the hippocampus, which may affect learning/memory as seen by the "saw-shaped" learning curve in the water maze, and may cause neurons to become highly vulnerable to other neuronal insults (e.g. glutamate, a ubiquitous neurotransmitter). This may resemble a pathological scenario in the brains of Alzheimer's patients, such that neurons that are adjacent to neuritic plaques with heavy glutaminergic inputs may be at high risk for degeneration.

The implications of this research are far-reaching; however, a replication is necessary to confirm the verity of these results. In addition, the full length peptide, ßAP(1-40), should be investigated for its effects on learning/memory in a similar context to the present study. Since *in vitro* evidence suggests that ßAP(25-35) works via a calcium-mediated mechanism, future studies should test this hypothesis by the use of calcium channel blockers (e.g. nimodipine, dantrolene, etc.) or calcium chelators (e.g. EGTA) *in vivo* to protect

neurons from the effects of βAP(25-35) and/or ibotenic acid. Investigation in this direction may lead to a possible treatment for Alzheimer's disease.

References

1. Allrispe, N., Rojas, E., Pollard H.B. (1993). Alzheimer disease amyloid ß protein forms calcium channels in bilayer membranes: Blockade by tromethan and aluminum. Proc. Natl. Acd. Sci. USA., 90, 567-571.

2. Frandsen, A., Schousboe, A. (1993). Excitatory Amino Acid-Mediated Cytotoxicity and Calcium Homeostasis in Cultured Neurons. Journal of Neurochemistry, 1202-2111.

3. Goedert, M., Spillantini, M.G., Jakes, R. (1991). Localization of the Alz-50 epitope in recombinant human microtubule-associated protein tau. Neuroscience Letters, 126, 149-154.

4. Handelmann, G.E., Olton, D.S. (1981). Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training. Brain research, 217, 41-58.

5. Hu, J., El-Fakahany, E.E. (1993). ß-amyloid 25-35 Activated Nitric Oxide Synthase in a Neuronal Clone. In press to Science.

6. Hyman, B.T., Hoesen, V.G.W., Wolozin, B.L., Davies, P.B., Kromer, L.J., Damasio, A.R. (1988). Alz-50 Antibody Recognizes Alzheimer-Related Neuronal Changes. Ann. Neurol., 23, 371-379.

7. Iqbal, K. (1991). Prevalence and Neurobiology of Alzheimer's Disease: Some Highlights. In D.R.C.M.K. Iqbal B. Winblad, and H.M. Wisniewski (Eds.), Alzheimer's disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies (pp. 1-5). New York: Wiley.

8. Joseph, R., Han, E. (1992). Amyloid beta-protein fragment 25-35 causes activation of cytoplasmic calcium in neurons. Biochm. Biophys. Res. Commun., 184(3), 1441-1447.

9. Kang, J., Lemaire, H.G., Untebeck, A., Salbaum, J.M., Master, C.L., Grzesckik, K.H., Multhaup, G., Beyreuther, K., Mullerhill, B. (1987). The Precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature, 325, 733-736.

10. Koh, J., Yang, L.L., Cotman, C.W. (1990). ß-Amyloid protein increases the vulnerability of cultured cortical neurons to excitotoxic damage. Brain Research., 533, 315-320.

11. Kowall, N.W., Beal, M.F., Busciglio, J., Duffy, L.K., Yankner, B.A. (1991). An in vivo model for the neurodegenerative effects of ß-amyloid and protection by subtance P. Proc. Natl. Acd. Sci. USA. 88, 7247-51.

12. Lee, V.M., Brian, B.J., Otvos, L., Trojanowski, J.Q. (1991). A68: A Major Subunit of Paired Helical Filaments and Derivatized Forms of Normal Tau. Science, 251, 675-78.

13. Masliah, E., Terry, R.D., Mallory, M., Alford, M., Hansen, L.A. (1990). Diffuse Plaques Do Not Accentuate Synapse Loss in Alzheimer's Disease. American Journal of Pathology, 137(6), 1293-1297.

14. Mattson, M.P., Cheng, B., Davis D., Bryant, K., Lieberburg, I., Rydel R.E. (1992). ß-Amyloid Peptides Destabilize Calcium Homeostasis and Render Human Cortical Neurons Vulnerable to Excitotoxicity. The Journal of Neuroscience, 12(2), 376-389.

15. Pike, C.P., Walencewicz, A.J., Glabe, C.G., Cotman C.W. (1991). Aggregation-related toxicity of synthetic ß-amyloid protein in hippocampal cultures. European Journal of Pharmacology, 207, 367-368.

16. Pike, C.P., Walencewicz, A.J., Glabe, C.G., Cotman C.W. (1991). In vitro aging of ß-amyloid protein causes peptide aggregation and neurotoxicity. Brain Research, 5 5 3 , 311-314.

17. Podlisny, M.B., Stephenson, D.T., Frosch, M.P., Lieberburg, I., Clemens, J.A., Selkoe, D.J. (1992). Synthetic Amyloid ß-Protein Fails to Produce Specific Neurotoxicity in Monkey Cerebral Cortex. Neurobiology of Aging, 13, 561-567.

18. Price, D.P., Davis P.B., Morris, J.C., White, D.L. (1991). The Distribution of Tangles, Plaques and Related Immunohistochemical Markers in Healthy aging and Alzheimer's Disease. Neurobiology of Aging, 12, 295-312.

19. Rogers, J., Morrisson, J.H. (1985). Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease. J. Neurosci. 5, 2801-8.

20. Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., Deteresa, R., Hill, R., Hansen, L.A., Katzman, R. (1991). Physical Basis of Cognitive Alterations in Alzheimer's Disease: Synapse Loss Is the Major Correlate of Cognitive Impairment. Ann. Neurol., 30, 572-580.

21. Yankner, B.A., Duffy, L.K., Kirschner, D.A. (1990). Neurotrophic and Neurotoxic Effects of Amyloid ß Protein: Reversal by Tchykinin Neuropeptides. Science, 250, 279-82.

Figure Caption

Figure 1. Group differences in mean percent animals consistently entering arms over the sessions. Consistent entry was characterized as at least 1 entry per session for at least 3 sessions in 1 block (1 block=6 sessions).

Figure 2. Group differences in the mean number of arms entered over the blocks (1 block=6 sessions). Vertical lines indicate S.E.M. *$p<0.05$, **$p<0.001$ significantly different from the saline controls.

Figure 3. Group differences in the mean number of working memory incorrect errors (WMIE) committed over the blocks (1 block=6 sessions). A WMIE is an arm entry into an arm that the rat has already made a reference memory error in the same session. Vertical lines indicate S.E.M. *$p<0.05$ significantly different from the saline controls.

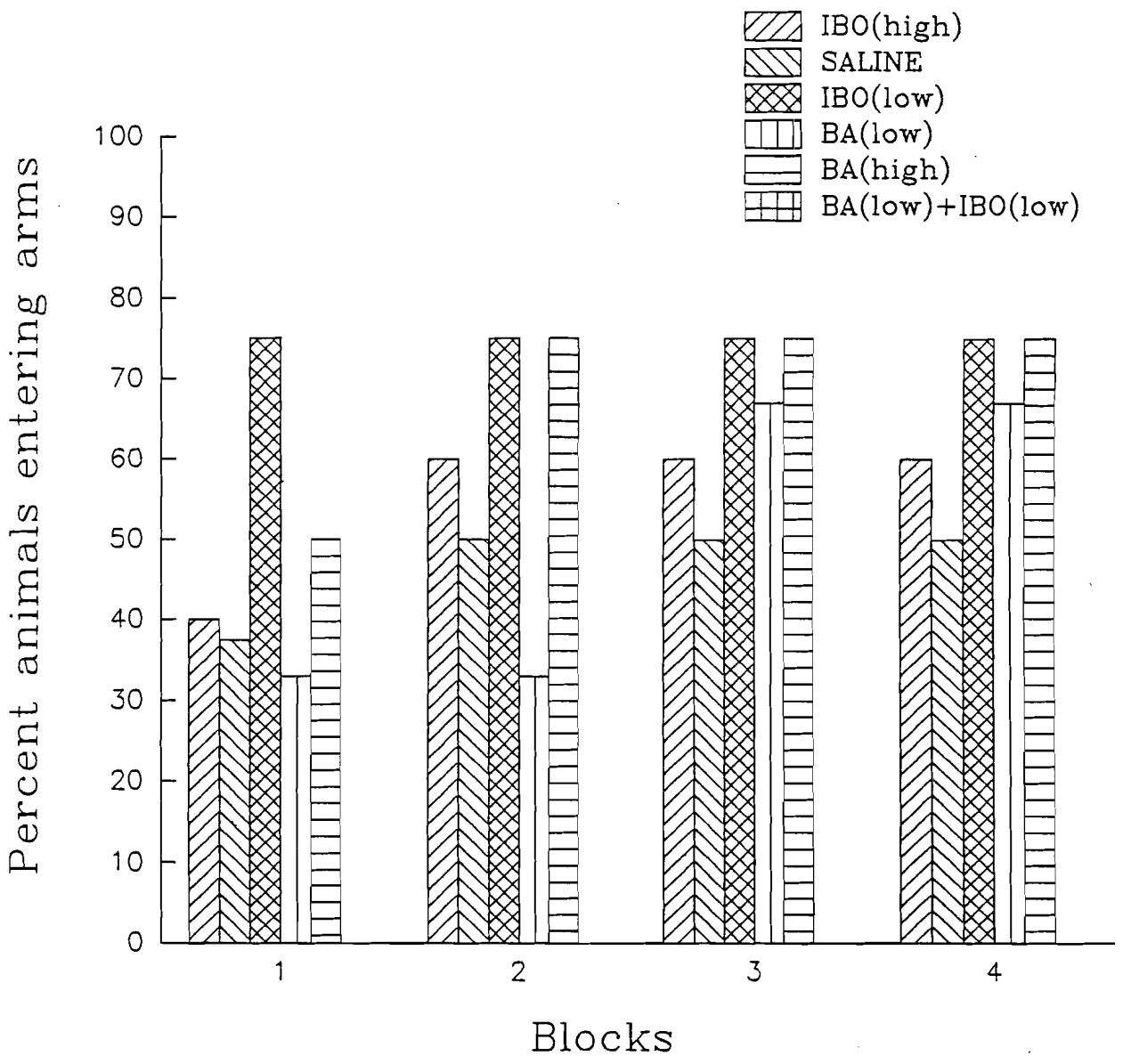
Figure 4. Group differences in the mean number of working memory correct errors (WMCE) committed over the blocks (1 block=6 sessions). A WMCE is an arm entery into an arm that the rat has already obtained food in the same session.

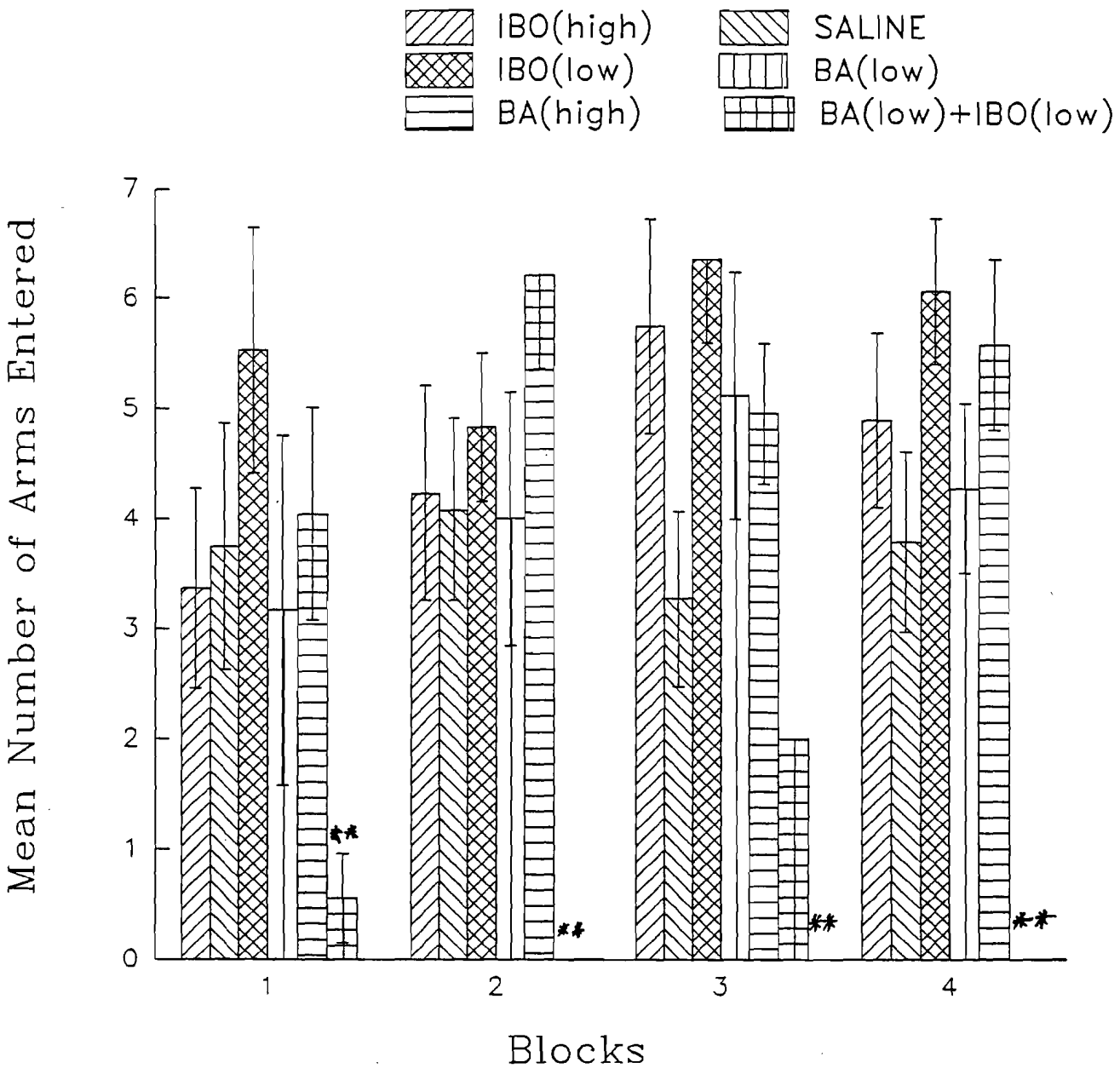
Vertical lines indicate S.E.M. *p<0.05 significantly different from the saline controls.

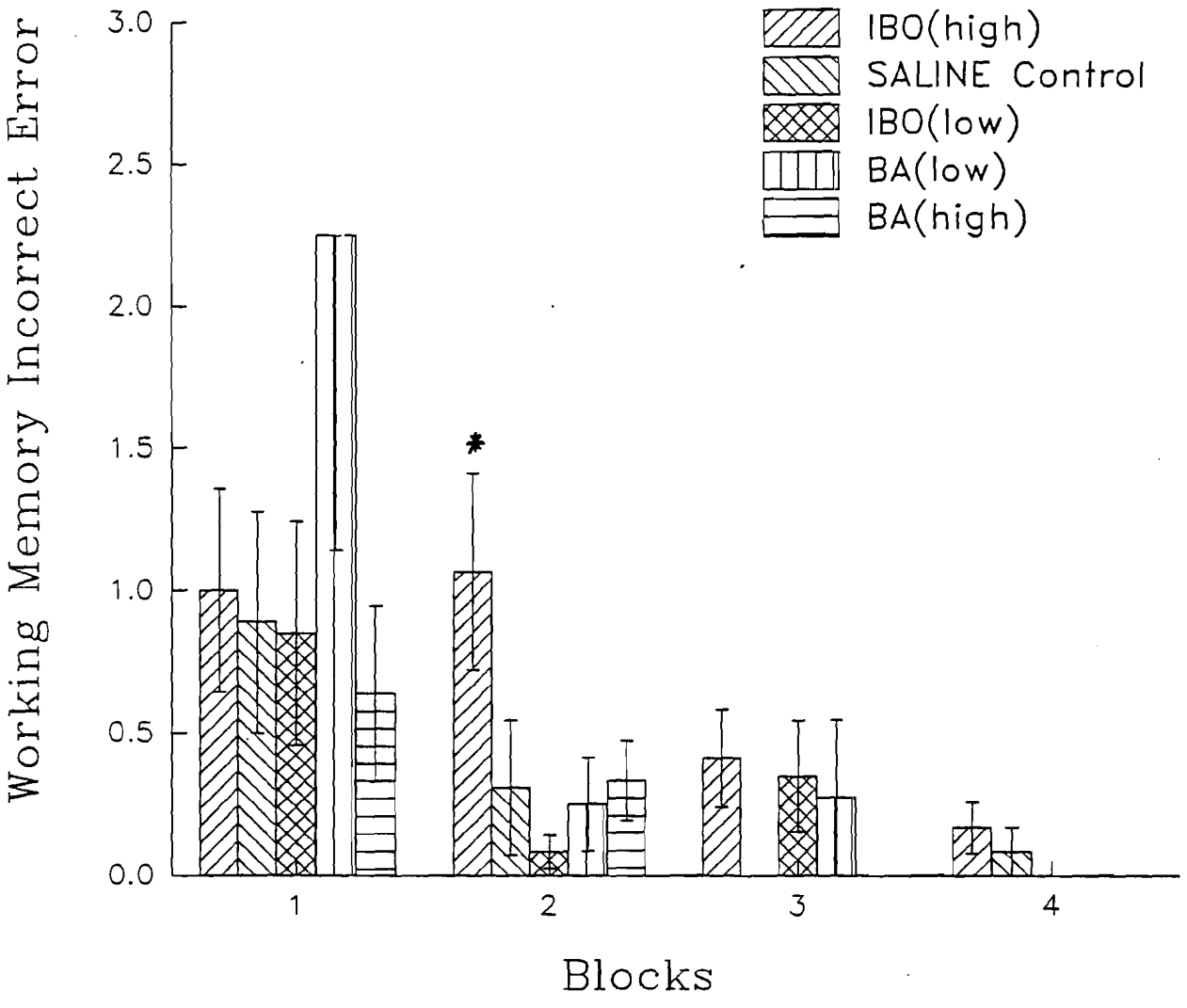
Figure 5. Group differences in mean choice accuracy (ChA) over the blocks (1 block=6sessions). ChA is defined as the number of correct arm entries prior to making an error. Vertical lines indicate S.E.M. *p<0.05 significantly different from the saline controls.

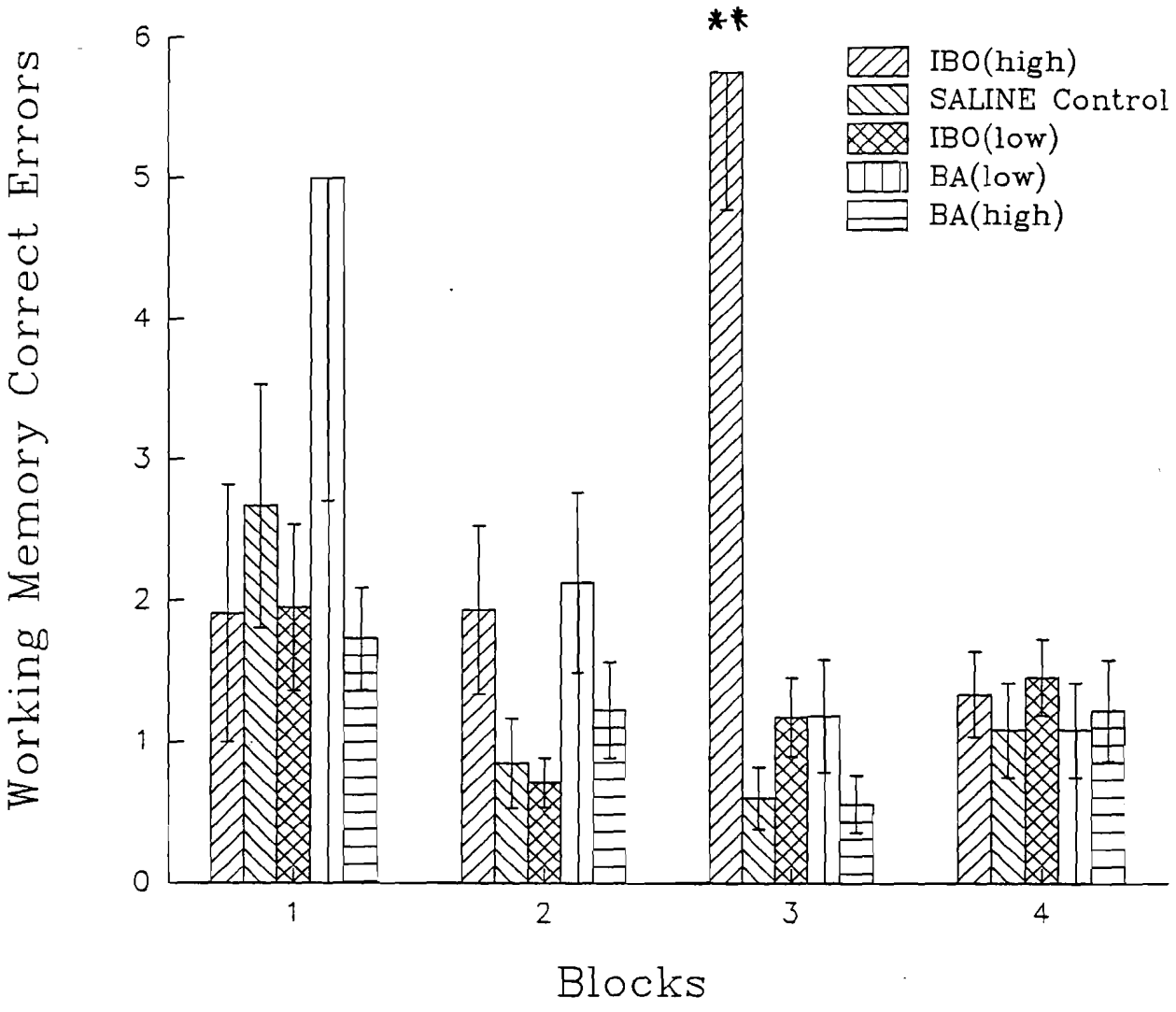
Figure 6. Differences in mean number of revolutions made in the treadmill motor activity test in 5 minutes between the rats that ran the RAM and those that did not. Vertical lines indicate S.E.M. There is no significant difference at p=0.05 level.

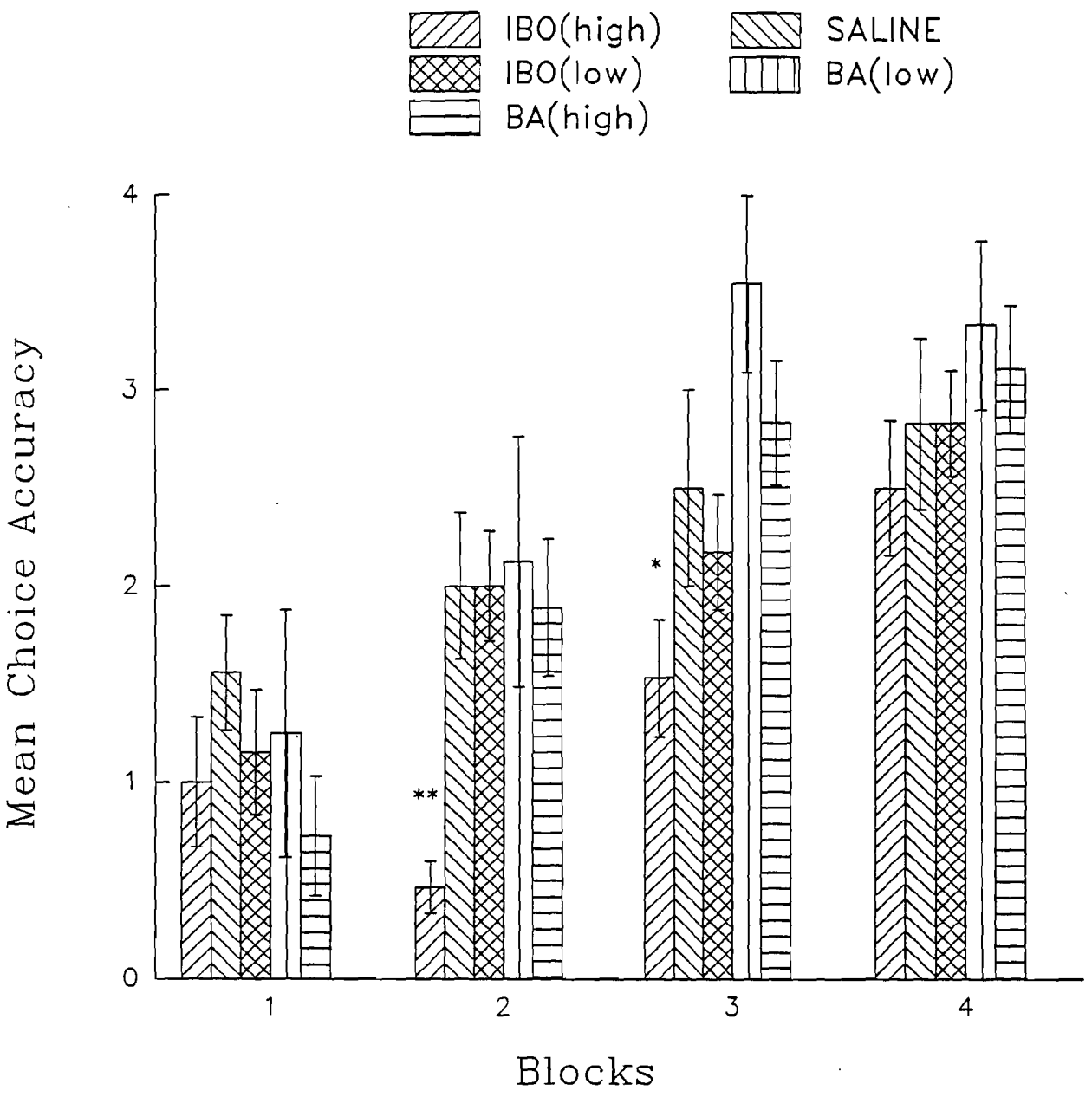
Figure 7. Group differences in mean escape latencies (seconds) over days (2 trials/day). *p<0.05 significantly different from the saline controls.

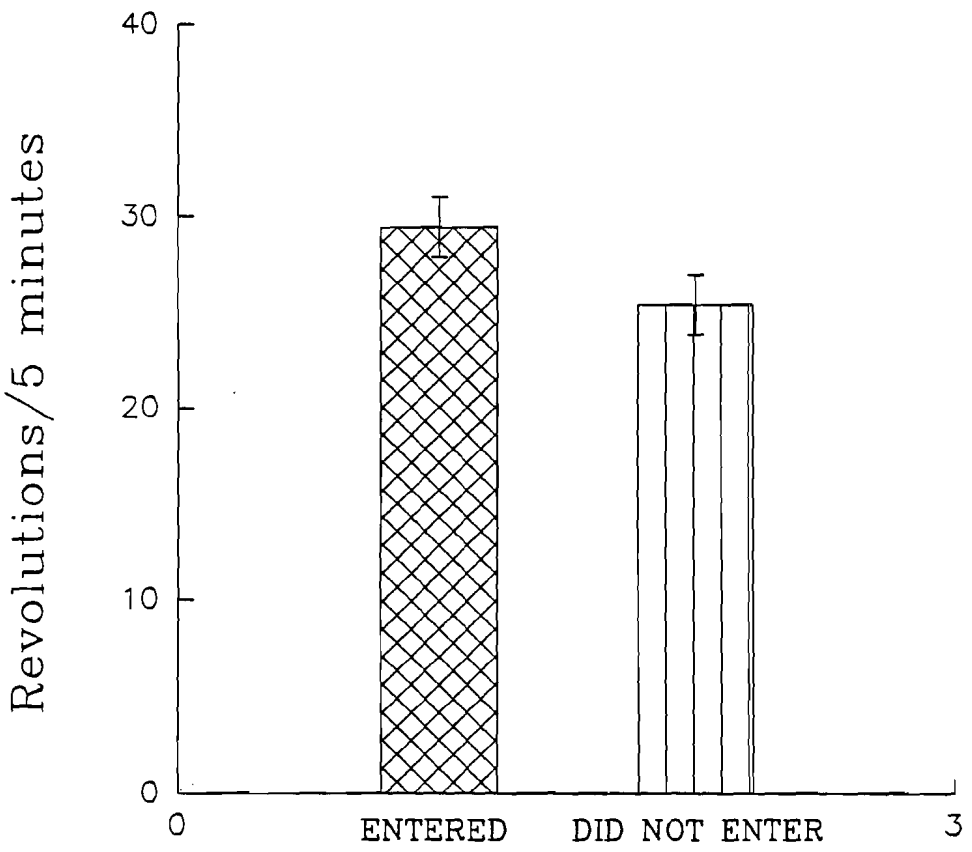

IBO(high)
SALINE
IBO(low)
BA(low)
BA(high)
BA(low)+IBO(low)
Percent animals entering arms
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Blocks

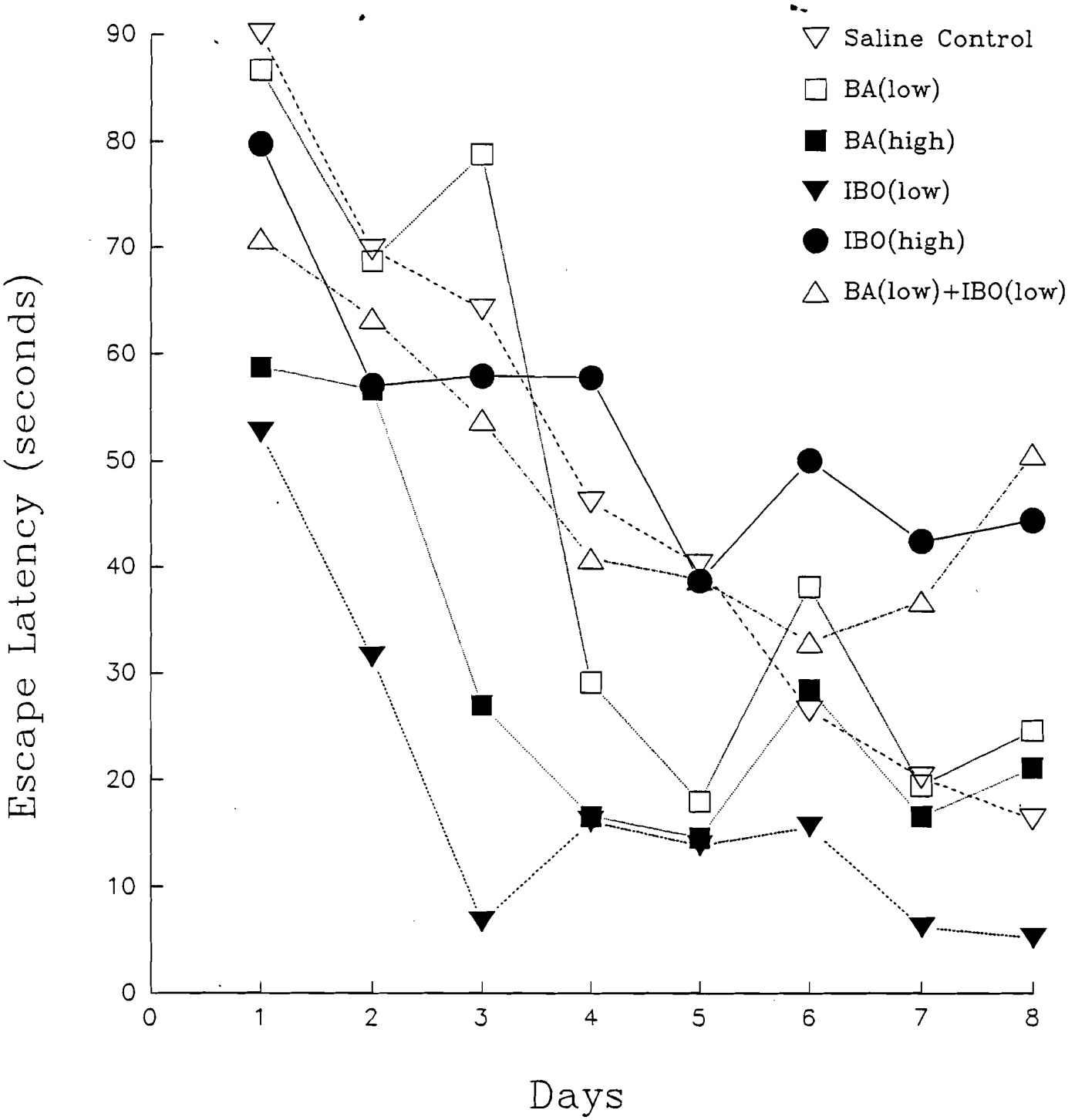

IBO(high)
IBO(low)
BA(high)
SALINE
BA(low)
BA(low)+IBO(low)
Mean Number of Arms Entered
0
1
2
3
4
5
6
7
**
**
**
**
1
2
3
4
Blocks


Working Memory Incorrect Error
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
Blocks
*
IBO(high)
SALINE Control
IBO(low)
BA(low)
BA(high)


Working Memory Correct Errors
0
1
2
3
4
5
6
**
IBO(high)
SALINE Control
IBO(low)
BA(low)
BA(high)
1
2
3
4
Blocks


IBO(high)
IBO(low)
BA(high)
SALINE
BA(low)
Mean Choice Accuracy
0
1
2
3
4
**
*
1
2
3
4
Blocks


Revolutions/5 minutes
40
30
20
10
0
0
ENTERED
DID NOT ENTER
3


Escape Latency (seconds)
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Days
Saline Control
BA(low)
BA(high)
IBO(low)
IBO(high)
BA(low)+IBO(low)