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Intrahippocampal Administration of Amyloid-β_{1-42} Oligomers Acutely Impairs Spatial Working Memory, Insulin Signaling, and Hippocampal Metabolism

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Abstract

Increasing evidence suggests that abnormal brain accumulation of amyloid-β_{1-42} ($A\beta_{1-42}$) oligomers plays a causal role in Alzheimer's disease (AD), and in particular may cause the cognitive deficits that are the hallmark of AD. *In vitro*, $A\beta_{1-42}$ oligomers impair insulin signaling and suppress neural functioning. We previously showed that endogenous insulin signaling is an obligatory component of normal hippocampal function, and that disrupting this signaling led to a rapid impairment of spatial working memory, while delivery of exogenous insulin to the hippocampus enhanced both memory and metabolism; diet-induced insulin resistance both impaired spatial memory and prevented insulin from increasing metabolism or cognitive function. Hence, we tested the hypothesis that $A\beta_{1-42}$ oligomers could acutely impair hippocampal metabolic and cognitive processes *in vivo* in the rat. Our findings support this hypothesis: $A\beta_{1-42}$ oligomers impaired spontaneous alternation behavior while preventing the task-associated dip in hippocampal ECF glucose observed in control animals. In addition, $A\beta_{1-42}$ oligomers decreased plasma membrane translocation of the insulin-sensitive glucose transporter 4 (GluT4), and impaired insulin signaling as measured by phosphorylation of Akt. These data show *in vivo* that $A\beta_{1-42}$ oligomers can rapidly impair hippocampal cognitive and metabolic processes, and provide support for the hypothesis that elevated $A\beta_{1-42}$ leads to cognitive impairment via interference with hippocampal insulin signaling.

Keywords

Amyloid; glucose; glucose transporter type 4; hippocampal; insulin; memory

INTRODUCTION

Alzheimer's disease (AD) is characterized by progressive impairment in cognitive function, decreased brain glucose metabolism, and both cortical and limbic amyloid deposition (in addition to other pathologies such as abnormal tau phosphorylation). Several recent imaging studies showed that brain glucose hypometabolism is correlated with total amyloid-β (Aβ) levels [1, 2], and the cognitive deficits caused by acute reductions in brain glucose

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metabolism are well known [3–13]. Abnormal accumulation of $A\beta_{1–42}$ is considered a causative agent in the pathologies of AD [14]; recently, attention has focused specifically on oligomeric $A\beta_{1–42}$, which has deleterious effects on neuronal function *in vitro* [15–18]. However, it is unknown whether oligomeric Aβ regulates brain glucose metabolism *in vivo*.

Chronic elevation of brain Aβ causes cognitive impairments and *in vitro* studies show extensive neurotoxicity of Aβ [19–24]. Neurotoxic $A\beta_{1–42}$ oligomers appear to act, at least in part, via inhibition of insulin signaling, which promotes reduced synaptic function [17, 18].$A\beta_{1–42}$ oligomers are known to bind and inactivate insulin receptors, which may not only impair synaptic function but potentially also impair brain glucose metabolism [25, 26]. Conversely, insulin can attenuate synaptic deficits caused by $A\beta_{1–42}$ oligomers [17, 18]. Importantly, we recently showed that hippocampal insulin signaling is a critical component of both mnemonic processing and local metabolic regulation [27]. Hence, interference with insulin signaling may be the mechanism by which Aβ oligomers impair neural function. One component of this action may be impairment of translocation of the insulin-sensitive glucose transporter GluT4 to the plasma membrane of hippocampal neurons. GluT4 is heavily expressed in the hippocampus [28–30] and has been suggested as a mediator of on-demand glucose supply during memory processing [28, 31, 32]; provision of glucose to the hippocampus is well-established to regulate mnemonic performance [33], and hippocampal glucose transport deficits may lead to hippocampal hypometabolism during AD [34–36]. Because $A\beta_{1–42}$ oligomers impair the phosphoinositide 3-kinase (PI3K) pathway [16, 17, 37, 38], which directly regulates GluT4 translocation in the CNS and periphery [30, 39, 40], we hypothesized that $A\beta_{1–42}$ oligomers would impair translocation of GluT4.

A further link between insulin signaling and Aβ is suggested by the comorbidity seen between AD and type 2 diabetes mellitus, a disease characterized by impaired insulin sensitivity [13, 41–44]. Postmortem analysis of brain tissue from AD patients shows several features associated with insulin resistance, prompting speculation that AD might be a type of brain diabetes [45]. Rodents placed on diabetogenic diets increase brain Aβ accumulation [46, 47], and have impairments to both hippocampally-mediated memory and hippocampalmetabolism [27].Collectively, these findings suggest that cognitive impairment in AD may be mediated through effects of Aβ on brain insulin signaling.

In the current study, we sought to determine the effects of acute intrahippocampal $A\beta_{1–42}$ oligomer administration on hippocampal metabolism, memory processes, and insulin signaling *in vivo*. Our primary hypothesis, that acute cognitive impairment would occur, was confirmed. Further, $A\beta_{1–42}$ oligomer administration prevented the task-associated dip in hippocampal extracellular fluid (ECF) glucose seen in untreated animals, attenuated hippocampal insulin signaling, and lowered hippocampal GluT4 translocation. These data provide support for the hypothesis that $A\beta_{1–42}$ oligomer accumulation leads to cognitive decline and hypometabolism because of interference with central insulin signaling, and suggests that impairment of GluT4 translocation may play a potentially critical role in the cognitive and metabolic deficits seen in AD patients.

METHODS

Subjects

Juvenile male Sprague-Dawley rats (Charles River, Wilmington, MA), approximately 300 g at time of arrival, were housed in pairs on a 12:12 h light:dark schedule with food and water available *ad libitum*. Following surgery, rats were housed singly. Rats were given at least one week to acclimate prior to any surgery or testing, during which time they were handled extensively. Rats were randomly assigned to one of three groups: vehicle control, scrambled $A\beta_{42\text{-}1}$, or $A\beta_{42\text{-}1}$ oligomers. Each rat was used only once. All procedures were approved by the University at Albany Institutional Animal Care and Use Committee.

Surgeries

Rats were anaesthetized with isoflurane and a single microinjection (Plastics One) or dual mD/microinjection cannula (BASi Cat. No. MD-2250) was stereotaxically implanted in the dorsal hippocampus using aseptic surgical technique. For microinjection cannula, the nose bar was set at 4.6 mm above the interaural line. Cannulae coordinates were 5.6 mm posterior to bregma, +4.6 lateral, and 3.3 ventral from dura. For experiments involving mD/microinjections, cannulae were implanted at 5.6 mm posterior to bregma, +5.0 lateral, and 3.0 ventral from dura. The difference in cannullae placement was to account for the 4 mm probe used in microdialysis experiments. Rats received acetaminophen in their drinking water following surgery and were allowed a two-week long recovery period prior to testing.

Treatment preparation

Soluble oligomers were made from synthetic human $A\beta_{1–42}$ using a slight modification of the procedure of Lambert et al. [20, 48]. $A\beta_{1–42}$ or scrambled $A\beta_{1–42}$ was reconstituted with ice-cold hexafluoroisopropanol (HFIP) and allowed to evaporate overnight in nonsiliconized microcentrifuge tubes. The next day, tubes were dried by vacuum centrifugation to remove remaining HFIP, leaving monomeric Aβ. Tubes were then stored over dessicant at −80°C until oligomer preparation. On the day prior to testing, stock preps were reconstituted in anhydrous DMSO and brought to 100 μMin either artificial extracellular fluid (aECF; 153.5 mM Na, 4.3 mM K, 0.41 mM Mg, 0.71 mM Ca, 139.4 mMCl, buffered at pH 7.4) or F12 medium without phenol red (Sigma), incubated at 4°C for 24 h, centrifuged for 14,000 g at 4°C for 10 min, and the supernatant collected for use as oligomers for animal treatment. Preparation of the scrambled control peptide (synthetic $A\beta_{42\text{-}1}$) and vehicle control followed the same protocol. All treatments were filtered with a 0.2 μm filter prior to microinjections to prevent clogging of the 40 μm pore of the dual mD/microinjection cannulae. Oligomerization was verified by silver staining using a commercially available kit (Pierce). Silver staining represents a quick and efficient way to folding states of isolated proteins following SDS-PAGE. It was previously found that $A\beta_{1–42}$ oligomers are SDS stable and detectable by the silver staining procedure using a 4–20% SDS-PAGE gel under non-denaturing conditions [20, 48]. Preparations of $A\beta_{1–42}$ oligomers in both aECF and F12 medium, but not scrambled $A\beta_{42\text{-}1}$, contained prominent bands consistent with the known molecular weights of monomers, dimers, and trimers, respectively [20, 48].

Behavioral testing

To assess spatial working memory we used spontaneous alternation (SA) in a 4-arm plus-maze. Rats perform this task using spatial working memory to remember the least-recently visited arms. Animals are placed into the maze and allowed to explore freely for 20 min and will spontaneously tend to visit the least-recently visited arm, using spatial working memory to recall arm-visit history; alternation scores are calculated by dividing the percentage of alternations (defined as a visit to each of the 4 arms within each span of 5 consecutive entries), with chance performance being 44%. Others and we have used this task extensively over the past decade to examine brain metabolism, insulin signaling, and spatial working memory in rats [27, 49–52]. Data are the combination of two independent replications.

Microinjections and in vivo microdialysis

Microdialysis was performed as previously described [53], with samples collected before, during, and after microinjection and subsequent behavioral testing. On the day of testing, a fresh 4 mm probe (BASi Cat. No. MD-2264) was placed into the guide cannula and the animal was allowed to acclimate for at least 1 h while perfused with aECF including 1.25 mM glucose at a rate of 1.5 μl/min. Perfusate was collected in 20-min samples. Two baseline samples were collected prior to microinjection. Microinjections were administered at a rate of 0.125 μl/min over 4 min for a total volume of 0.5 μl, 10 min prior to behavioral testing. This volume corresponds to approximately 200 ng of either $A\beta_{1–42}$ oligomers or scrambled $A\beta_{42\text{-}1}$. Rats in the no maze condition were left in the control chamber during the ‘testing’ period. Glucose and lactate were measured in a CMA600 analyzer (Stockholm, Sweden). Sample measurements were corrected for *in vivo* probe recovery using a zero-net-flux plot for ECF glucose measurements under the same experimental conditions.

Histology

Immediately following experimentation, rats were sacrificed. Probe placement was verified using Nissl staining on coronal sections in half of the subjects to ensure consistency. In the other half of subjects, whole hippocampi were removed, frozen on dry ice, and stored at −80°C until plasma membrane extraction. In animals that underwent mD, all brains were checked by Nissl staining for probe placement. Data from animals that had misplaced cannulae or evidence of excessive inflammation were excluded ($n = 4$).

Cell fractionation

Plasma membrane (PM) fractions were collected using a commercially available kit (Biovision). Whole hippocampi were homogenized with a Polytron hand-held electric homogenizer in 3 volumes of (1 mg/3 μl) lysis buffer with lyophilized protease inhibitors (Bio-vision). Following the initial homogenization 30 μl of the total lysate was added to 200 μL ice-cold RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate,1%Triton) containing fresh protease and phosphatase inhibitors (Pierce) and stored at −80°C until protein quantification. For the remaining lysate, PM separation proceeded according to the kit’s instructions.

Immunoblotting

Protein concentration was determined using a commercially available kit for the bicinchoninic assay (Pierce). 10–20 μg of protein was placed in sample buffer in denaturing and reducing conditions. Samples tested for total protein content were heated at 95°C for 5 min prior to sample loading. Samples analyzed for plasma membrane associated proteins were not heated prior to gel loading. Samples were resolved on 8% or 12% SDS polyacrylamide minigels (Pierce or Bio-Rad) and transferred onto PVDF membranes (Bio-Rad) followed by detection with appropriate primary antibodies. Antibodies used were anti-rabbit directed against GluT4 (Millipore Cat. No. 07-1404), GluT1 (Abcam Cat. No. ab652.), GluT3 (Abcam Cat. No. ab41525), phosphorylated Akt at serine 473 (Cell Signaling Technology Cat. No 4060L), and total Akt (Cell Signaling Cat. No. 4691L). Monocolonal anti-mouse directed against Na+/K+ ATPase (Abcam Cat. No. ab7671) or β-actin (Sigma Cat. No. A2228) were used as loading controls for PM and total protein samples, respectively. Following wet-transfer at 350 mA in the cold specify for 60 min, PVDF membranes were blocked for 1 h with 5% milk or 5% BSA in tris-buffered saline containing 0.1% tween-20. Membranes were incubated with primary antibodies in blocking solution overnight at 4°C in conditions optimized for immunodection by each antibody. Secondary incubation in biotin-conjugated goat anti-mouse (Pierce) or goat anti-rabbit (Vector) IgG was followed by incubation in HRP strepdavidin (Pierce) for enhanced immunoreactivity, both for 1 h at room temperature. Immunoblots were developed with chemiluminscent detection using the SuperSignal West Pico Chemiluminescent Kit (Pierce). Images were taken in a Bio-rad ChemiDoc XRS Image Analyzer. Membranes were then stripped with Restore Plus Stripping Buffer (Pierce) and reprobed for loading controls or total protein content. Band intensity was measured by densitometry in ImageQuant software and normalized to loading controls or total protein content. To control for between gel variations in band intensity, vehicle control values were normalized to 100% and scrambled $A\beta_{42\text{-}1}$ and $A\beta_{1–42}$ samples were analyzed as percents of control values.

Statistical analyses

Statistics are reported as means ± standard error of the mean. For statistical comparisons of two groups, we used the student's *t* test, and for comparisons of more than two groups we used one-way ANOVA followed by Tukey's post hoc test. To test for the equality of group variances assumption the Levene test was used in all analyses. An α level of 0.05 was set for significance. All statistical analyses were made in SPSS version 17.

RESULTS

Intrahippocampal administration of $A\beta_{1–42}$ oligomers impaired spatial working memory

A single administration of $A\beta_{1–42}$ oligomers to the left hippocampus significantly impaired SA behavior, tested 10 min later, when compared to vehicle or scrambled $A\beta_{42\text{-}1}$ control groups (Fig. 1A). Scrambled $A\beta_{42\text{-}1}$ did not affect SA behavior. There was no significant difference in the number of arm entries made between groups, a measure which controls for nonspecific effects of treatment on motor activity or motivation (Fig. 1B).

$A\beta_{1–42}$ oligomers prevented the task-associated dip in extracellular glucose during the working memory task

Rats in the scrambled Aβ and vehicle control groups showed an approximately 30% dip in hippocampal ECF glucose, highly consistent with our previous work [51, 53, 54], which has shown increased hippocampal metabolic demand during SA testing. No such dip was seen during maze testing in rats treated with $A\beta_{1–42}$ oligomers (Fig. 2A). $A\beta_{1–42}$ oligomer treatment did not affect the rise in lactate levels that occurred during SA testing (Fig. 2B).

$A\beta_{1–42}$ oligomers did not affect hippocampal ECF glucose in the absence of cognitive load

Animals were treated and handled identically to maze-tested animals, but were returned to their home chamber rather than being placed into the maze. Hippocampal ECF glucose was unchanged following administration of either vehicle control or $A\beta_{1–42}$ oligomers (Fig. 3.

$A\beta_{1–42}$ oligomers attenuated Akt activity and plasma membrane levels of the insulin-sensitive glucose transporter GluT4

Treatment with $A\beta_{1–42}$ oligomers caused a significant reduction in the ratio ofPMto total GluT4, but did not alter total GluT4 (Fig. 4, suggesting interference with translocation rather than synthesis or degradation, as would be expected from the acute treatment. Akt, a downstream intermediate in the phosphatidyl-inositol-3-kinase (PI3K) signaling cascade, can be phosphorylated at either serine 473 ($pAkt^{ser473}$) or threonine 408. Phosphorylation at serine 473 is necessary for full activation of Akt and signaling to downstream protein activity, including GluT4 translocation to the plasma membrane (64), and we hence examined the effect of $A\beta_{1–42}$ oligomer administration on $pAkt^{ser473}$. Our western blot analyses revealed a significant reduction in $pAkt^{ser473}$ (normalized to total Akt) following $A\beta_{1–42}$ oligomer treatment, but no significant effect on total Akt protein (Fig. 5).

$A\beta_{1–42}$ oligomers had no effect on GluT1 or GluT3

We examined both isoforms of GluT1 and found no change in the ratio of plasma membrane to total expression following treatment with $A\beta_{1–42}$ oligomers, nor in plasma membrane or total expression of GluT1. Similarly, expression of neuronal GluT3 was not significantly affected by treatment with $A\beta_{1–42}$ oligomers, There was no difference in total GluT1 or GluT3 following $A\beta_{1–42}$ oligomer treatment (Fig. 6).

DISCUSSION

The results of this study show that $A\beta_{1–42}$ oligomers acutely impair both cognitive and metabolic processes within the hippocampus, with these effects likely being mediated (at least in part) through interference with insulin-regulated glucose transport and metabolism, a key modulator of hippocampal function. Attenuation of brain glucose metabolism is characteristic of AD; several studies have found decreased glucose transport and phosphorylation in the brains of AD patients [35, 36, 55]. In the periphery, insulin plays a key role in glucose uptake through regulation of GluT4 activity. Because insulin regulates glucose uptake in discrete brain areas including the hippocampus [27, 56], which expresses both insulin receptors and GluT4 at high levels [29–32], it is reasonable to suggest that insulin may support neuronal functioning by increasing local glucose metabolism. Indeed,

we previously showed, using *in vivo* microdialysis, that intrahippocampal insulin delivery, at a dose that enhanced spatial working memory, caused a decrease in hippocampal extracellular glucose and a concomitant increase in lactate, consistent with increased local glycolysis [27, 57]. $A\beta_{1–42}$ oligomers' role as synaptic pathogens may stem, at least partially, from their detrimental effects on insulin signaling, which could lead to attenuated glucose metabolism and synaptic activity. In the present study, delivery of $A\beta_{1–42}$ oligomers to the hippocampus had effects very similar to those produced by other memory-impairing agents such as morphine: worsened spatial memory accompanied by an absence of task-associated dip in ECF glucose [58]. Previous studies of Aβ on cognitive impact have largely focused on chronic effects following up to several weeks of treatment, generally given i.c.v. and hence affecting much of the brain [19–24]. The fact that $A\beta_{1–42}$ oligomers impair cognitive and metabolic processes very rapidly after intrahippocampal administration is consistent with the immediate impact of $A\beta_{1–42}$ oligomers on insulin-mediated PI3K activity previously shown [15–18], and is potentially necessary for the hypothesis that amyloid-induced cognitive impairments occur due to blockade of insulin signaling: we previously showed that cognitive deficits emerge within 10 min of hippocampal insulin signaling blockade [27].

We also show that administration of $A\beta_{1–42}$ oligomers acutely impairs translocation of the insulin-sensitive glucose transporter GluT4, as well as impairing PI3K signaling; as far as we know, this is the first *in vivo* confirmation that acute elevation in $A\beta_{1–42}$ oligomers regulates $pAkt^{ser473}$. GluT4 expression is abundant throughout the hippocampus. Moreover, GluT4 immunoreactivity colocalizes with cholinergic markers [59]. These data are thus relevant for AD pathology, which shows extensive damage to synapses and cholinergic neurons [15, 60]. Although some studies have examined glucose transporters in postmortem analysis of the brains of human AD patients, relatively little is known about the physiological role of hippocampal GluT4 during AD. Measurements of total protein have suggested that there may be little or no change in the total amount of GluT4, although total GluT1 and GluT3 were decreased [61, 62]. The present data illustrate the need for *in vivo* measurement of both total and plasma-membrane GluT4: we also found no change in total GluT4 protein, following Aβ administration, but the impact of $A\beta_{1–42}$ oligomers was clearly seen as a reduction in translocation to the plasma membrane (and hence a functional impairment). One might speculate that if anything, the brains of AD patients might show normal or slightly elevated total GluT4 protein, as possible compensation for impaired trafficking to the plasma membrane.

No effect on hippocampal metabolism was seen following administration of $A\beta_{1–42}$ oligomers in the absence of cognitive testing, suggesting that the effects of Aβ may be seen only at times of elevated cognitive demand. This pattern is highly consistent with a large literature showing that alterations in glucose supply to the hippocampus also affect function only under conditions of high cognitive load [58, 63, 64]. The metabolic data presented here are also consistent with our protein-level measurements of the impact of Aβ administration on glucose transporters: no effect was seen on the constitutively membrane-located transporters GluT1 or GluT3, but Aβ oligomers markedly impaired translocation of the insulin-sensitive transporter GluT4, which could provide additional neuronal glucose transport capacity when hippocampal mnemonic processes, of which insulin signaling is a

key component, are upregulated [27]. Additionally or alternatively, it is possible that $A\beta_{1–42}$ oligomers may impair glucose supply within the hippocampus through vascular effects. The fact that ECF lactate levels were not altered by Aβ administration, despite decreased glucose usage, might suggest that the impact of Aβ administration is at least in part on oxidative rather than non-oxidative metabolism; in that case, reduction in oxygen supply (presumably via impaired neurovascular coupling) might also contribute to the cognitive impairments observed. We are currently investigating whether hippocampal blood flow is altered following Aβ administration. Our data are consistent with PET studies in humans, where measurements taken at baseline (low cognitive load) in AD patients show that Aβ levels can be elevated without observable decrease in brain glucose metabolism [65, 66].

Previous studies found that impaired glucose metabolism and PI3K activity is associated with chronically increased Aβ levels [46, 67], but the current study demonstrates that even acute elevation in hippocampal $A\beta_{1–42}$ oligomers is sufficient to attenuate Akt phosphorylation and GluT4 translocation. These data are consistent with prior reports that $A\beta_{1–42}$ oligomers can directly attenuate the insulin-mediated PI3K pathway [37, 38]. Acute hyperinsulinemia, including intranasal administration of insulin, improves cognition in the elderly [68–70], possibly by increasing insulin-mediated glucose transport through GluT4 [71]. Activation of PI3K by insulin promotes α-secretase mediated cleavage of AβPP leading to the production of soluble AβPPα, which has neuroprotective properties [37]. Insulin is also a known regulator of Aβ degradation and clearance from the brain [72, 73]. Because $A\beta_{1–42}$ monomers and insulin compete for breakdown by insulin-degrading enzyme, which shows a stronger affinity for insulin than $A\beta_{1–42}$ monomers [72], chronic long-term hyperinsulinemia during insulin resistance may promote Aβ aggregation by preventing the breakdown of $A\beta_{1–42}$ monomers. Taken together with the present data, these studies offer further support for the suggestion that insulin and oligomeric Aβ mutually oppose, with intact insulin signaling protecting against Aβ toxicity but elevated Aβ impairing insulin signaling, consistent with the clinical finding that insulin resistance is a risk factor for AD [13, 27, 41–44, 46, 47]. It is possible, and indeed likely, that the effects of chronic $A\beta_{1–42}$ elevation on brain metabolism and cognitive function may vary across time; future studies aimed at clarifying the timeline along which $A\beta_{1–42}$ oligomers impair hippocampal function by various potential mechanisms may build on the present work.

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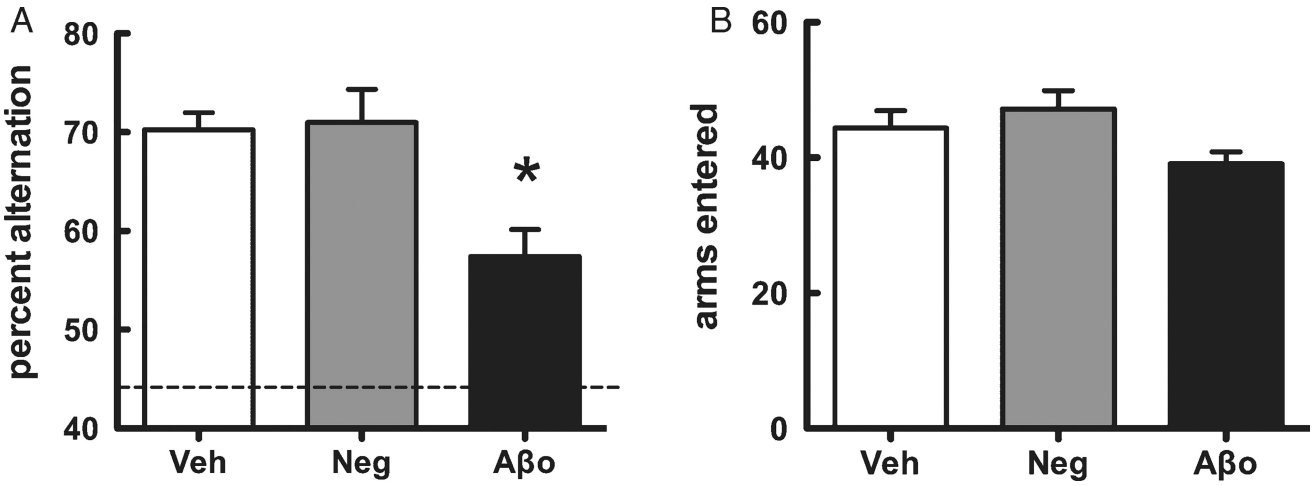

Fig. 1.

Spatial memory (spontaneous alternation performance, SA) following administration of $A\beta_{1–42}$ oligomers to dorsal hippocampus 10 min prior to behavioral testing. A) SA performance scores (mean ± SEM). Dotted line represents chance performance (44%). B) The number of arms entered during the SA task was not significantly different across treatment groups (mean ± SEM). *$p < 0.05$.

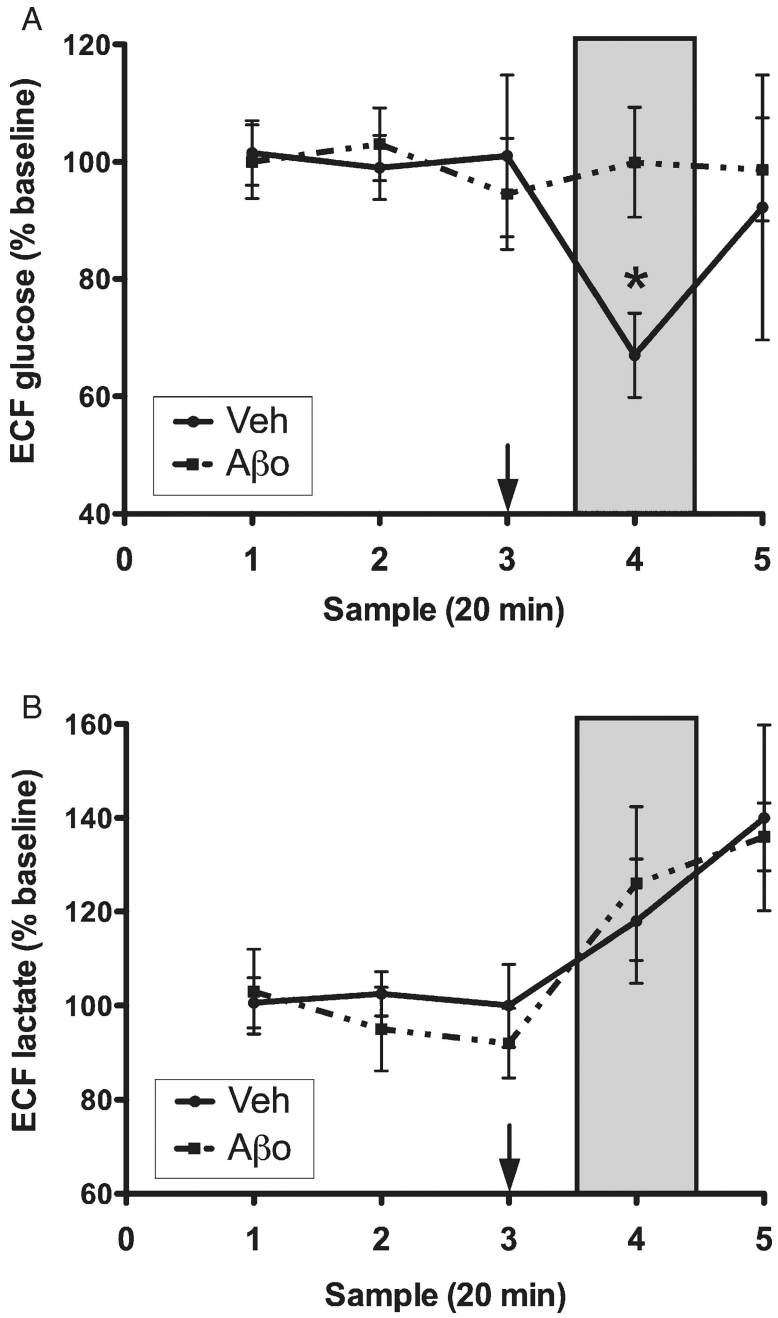

Fig. 2.

Hippocampal extracellular fluid (ECF) glucose (A) and lactate (B). Samples 1–3 are baseline measurements, sample 4 (shaded box) was collected during spatial memory testing, and sample 5 is post-maze. Treatment was administered 10 min prior to maze testing (arrow). Each data point is mean ± SEM. *$p < 0.05$.

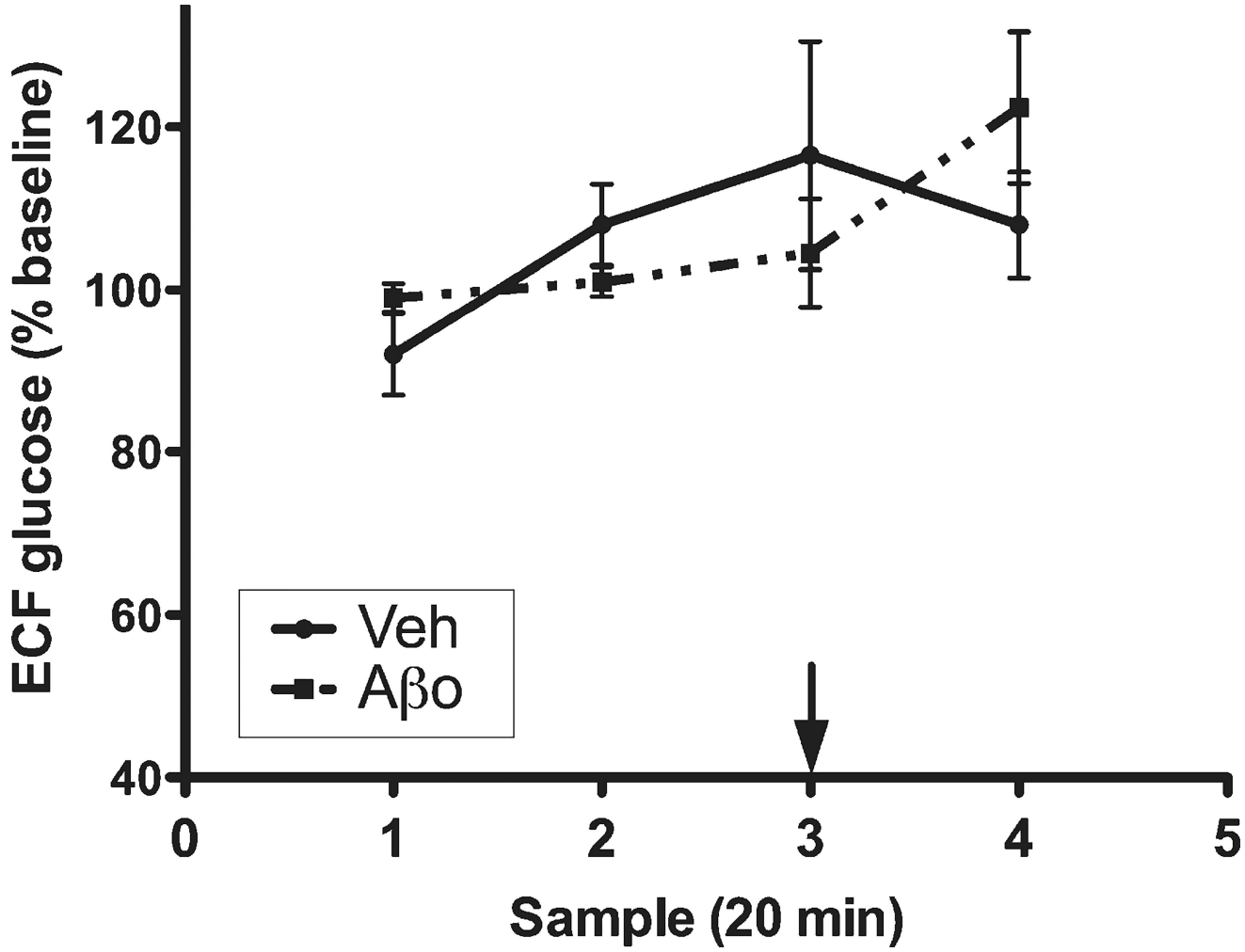

Fig. 3.
Hippocampal ECF glucose under control (no-testing) conditions. Samples 1–2 are baseline measurements. Treatment was administered 50 min into collection (arrow).

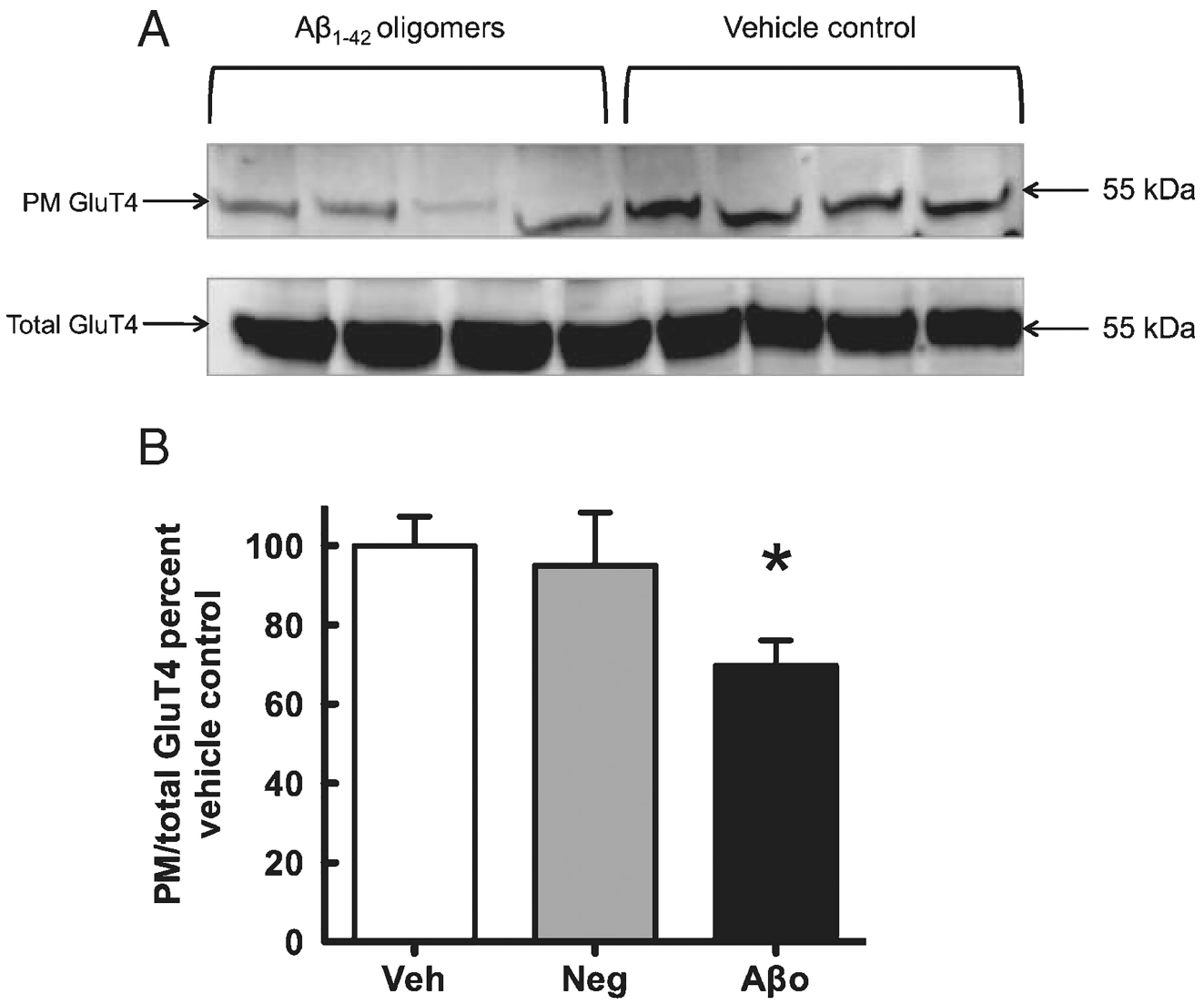

Fig. 4.

A) Representative western blot images for PM and total GluT4. B) Analysis of western blots comparing plasma membrane GluT4 to total GluT4. Vehicle control = Veh, $A\beta_{42\text{-}1}$ = Neg, $A\beta_{1–42}$ oligomers =Aβo. Veh normalized to 100% and compared to Neg and Aβo. Each data point is mean ± SEM. *$p < 0.05$.

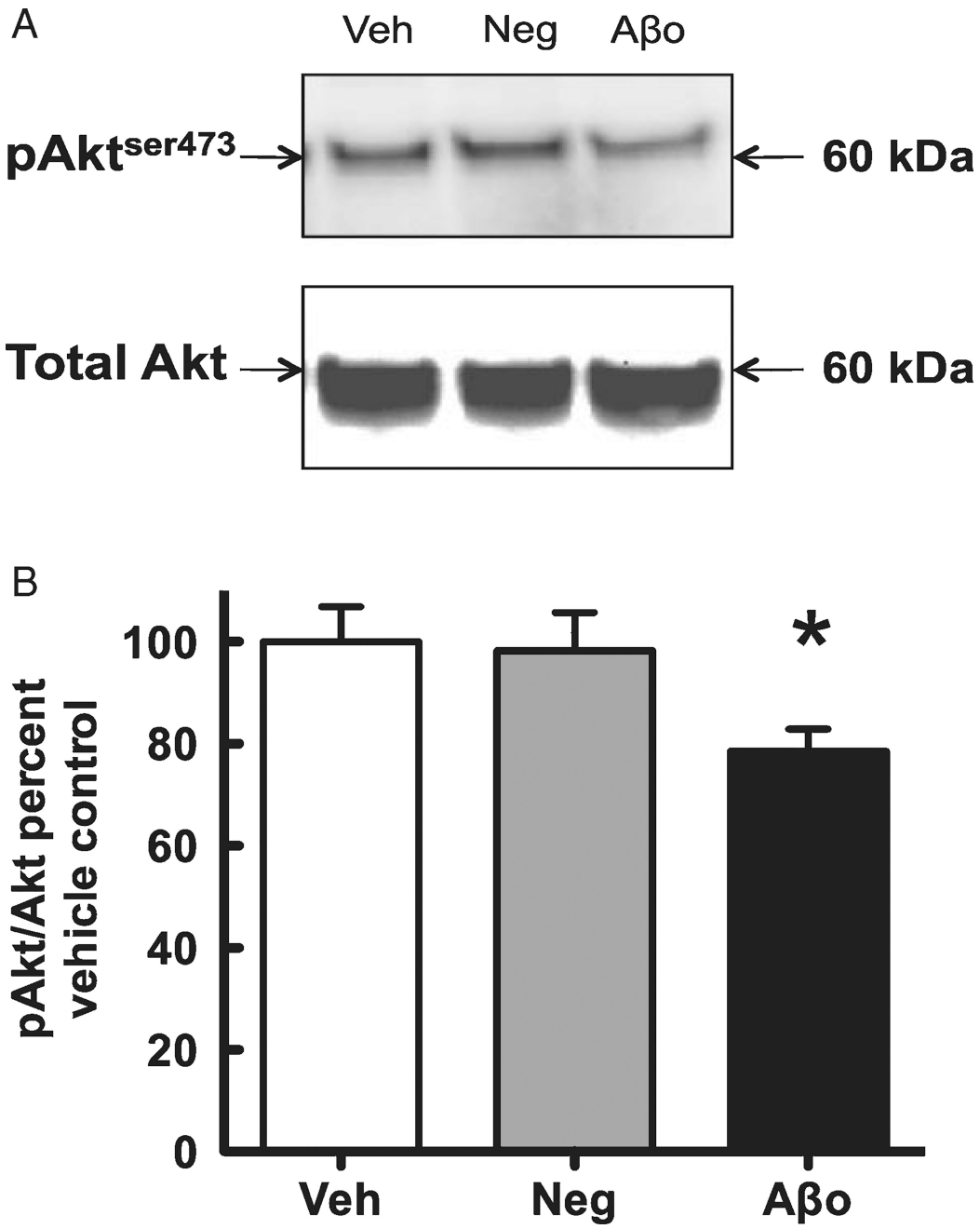

Fig. 5.

A) Representative western blot images for phosphorylated Akt at serine 473 (pAkt) and Akt. B) Analysis of western blots comparing pAkt to Akt. Vehicle control = Veh, $A\beta_{42\text{-}1}$ = Neg, $A\beta_{1–42}$ oligomers =Aβo.Veh normalized to 100% and compared to Neg and Aβo. *$p < 0.05$.

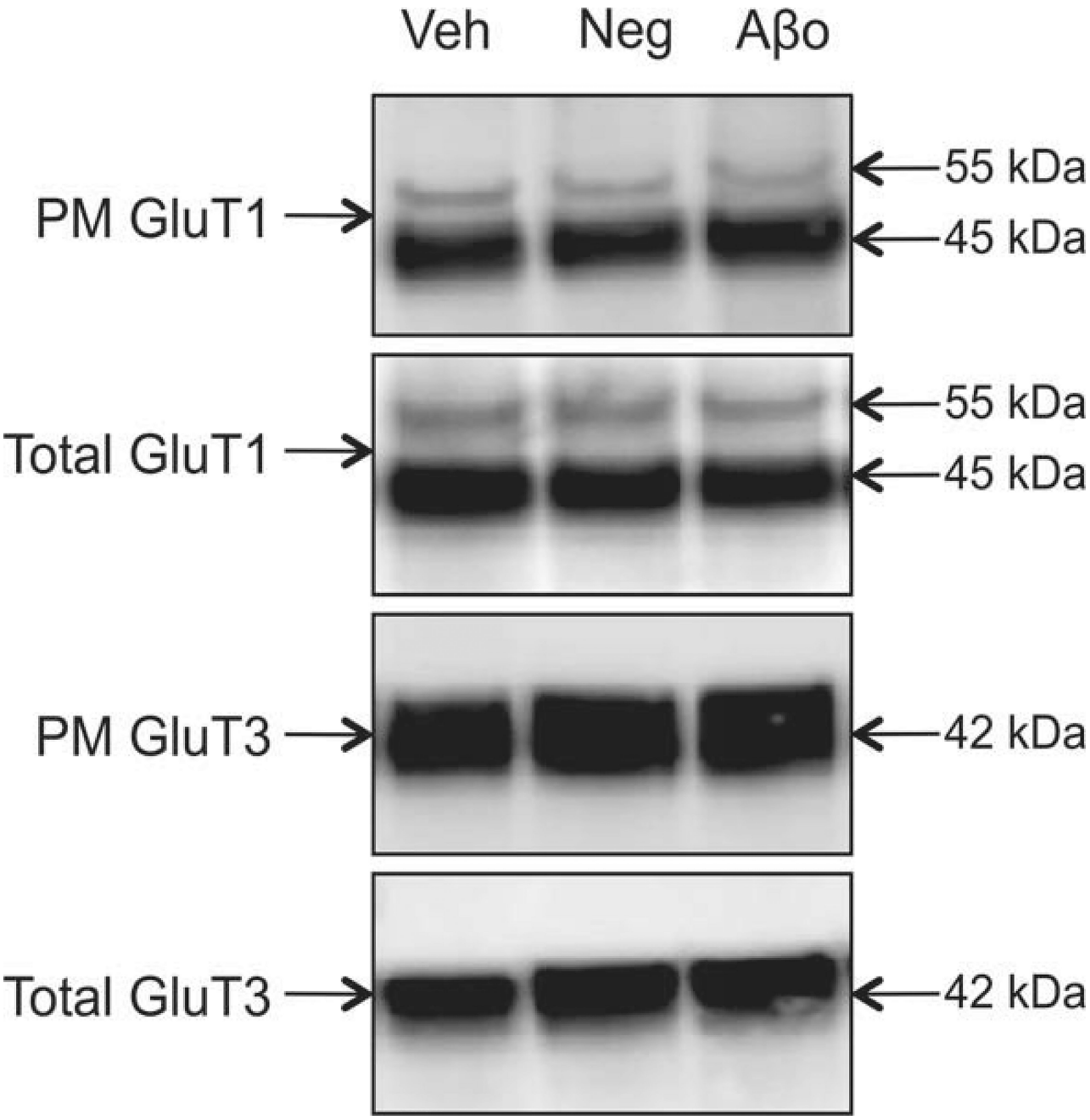

Fig. 6.

A) Representative western blot images for PM and total GluT1 and GluT3. There were no statistically significant differences between treatment groups. Vehicle control Veh, $A\beta_{42\text{-}1}$ = Neg, $A\beta_{1–42}$ oligomers = Aβo. Veh normalized to 100% and compared to Neg and Aβo. *$p < 0.05$.