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Abstract: Alzheimer's disease is a neurodegenerative disorder that affects mostly the elderly. The main histopathological markers are the senile plaques formed by amyloid- β peptide ($A\beta$) aggregates that can perforate the plasma membrane of cells, increasing the intracellular calcium levels and releasing synaptic vesicles that finally lead to a delayed synaptic failure. Several membrane proteins and lipids interact with $A\beta$ affecting its toxicity in neurons. Here, we focus on NMDA receptors (NMDARs) as proteins that could be modulating the association and neurotoxic perforation induced by $A\beta$ on the plasma membrane. In fact, our results showed that decreasing NMDARs, using enzymatic or siRNA approaches, increased the association of $A\beta$ to the neurons. Furthermore, overexpression of NMDARs also resulted in an enhanced association between NMDA and $A\beta$. Functionally, the reduction in membrane NMDARs augmented the process of membrane perforation. On the other hand, overexpressing NMDARs had a protective effect because $A\beta$ was now unable to cause membrane perforation, suggesting a complex relationship between $A\beta$ and NMDARs. Because previous studies have recognized that $A\beta$ oligomers are able to increase membrane permeability and produce amyloid pores, the present study supports the conclusion that NMDARs play a critical protective role on $A\beta$ actions in hippocampal neurons. These results could explain the lack of correlation between brain $A\beta$ burden and clinically observed dementia.

Keywords: Alzheimer's disease, amyloid-beta, glutamate, glycine receptor, hippocampal neurons, membrane damage, membrane pore, NMDA receptor

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder in the elderly [1]. AD manifests progressively with cognitive and behavioral impairments [2] characterized by loss of memory and learning [3]. One of the major histopathological markers of AD are the senile plaques formed by amyloid- β peptide ($A\beta$) aggregates [4]. These $A\beta$ aggregates produce a complex cascade of events that finally lead to synaptic failure and neuronal death [5].

A common hypothesis used to explain the toxicity induced by $A\beta$ is the formation of amyloid pores in the plasma membrane. Regarding this, several studies from our and other laboratories support the notion that membrane disruptions are induced by $A\beta$ [6–13]. Furthermore, $A\beta$ perforation allows the entry of small molecules and ions, such as calcium, into the cells [6, 7, 12]. This sustained calcium influx increases the release in synaptic vesicles, leading to a delayed synaptic failure produced by vesicle depletion [6–8].

It is now believed that A β association and toxic actions at the membrane level can be affected by the lipid and protein composition [9, 12, 14–18]. N-methyl-D-aspartate receptors (NMDARs) are excitatory ligand gated ion channels that have been described as important for some of the toxic effects induced by A β , similar to that observed with other membrane proteins like cellular prion, mGluR5, nicotinic receptor, and A β PP [9, 19–25]. The glutamate NMDA receptor is a tetramer composed of different subunit combinations (NR1, NR2A-D) allowing the influx of Na⁺ and Ca²⁺ ions into the neurons resulting in excitation [26, 27]. The relationship between AD and NMDARs is widely postulated [28, 29], but the mechanisms involving this relationship are not fully understood.

One of the strongest evidence for the clinical relevance of such interactions between A β and NMDARs in AD is that the NMDA receptor antagonist, memantine, is used clinically in the treatment of AD [28]. In fact, other NMDAR antagonists, like (+)MK-801, or the removal of extracellular Ca²⁺, reduced A β ₁₋₄₀-induced Ca²⁺ transients, NO production and neurotoxicity in cultured neuroblastoma cells [30]. Moreover, (+) MK-801 partially prevented the decrease in cell viability and the energy impairment induced by A β ₁₋₄₂ in HEK293 cells transiently expressing NR1/NR2A or NR1/NR2B subunits [29].

Regarding a potential interaction between A β and NMDAR, the data is controversial. While some authors indicate that the co-immunoprecipitation of A β dodecameric oligomers with NR1 and NR2A is evidence for their interaction [31], others have failed to detect binding of A β ₁₋₄₂ to any known regulatory sites on glutamate receptors [28]. Furthermore, recent data indicates that such effects of A β ₁₋₄₂ on NMDA receptors may be due to its binding to postsynaptic anchoring proteins such as PSD-95 or other membrane proteins like prion [21, 32, 33].

Here, in an attempt to clarify a potential role of NMDARs on A β ₁₋₄₂-induced neurotoxicity, we examined the ability of the peptide to associate to and disrupt plasma membranes, something that has not been studied until now. The results indicate that NMDARs are an important factor controlling A β neurotoxicity.

Materials and Methods

Primary cultures of rat hippocampal neurons

Hippocampal neurons were obtained from 18-day pregnant Sprague-Dawley rats and maintained for 10-14 days *in vitro* (DIV) as previously described [34]. All animals were handled in strict accordance with NIH guidelines and approved by the Ethics Committee of the Universidad de Concepción (Concepción, Chile).

Peptide preparation and storage

Human A β ₁₋₄₂ labeled with FAM (green fluorescence, A β -FAM) at its N-terminus, and unlabeled peptides were purchased from Anaspec (CA, USA). The preparation and storage were performed as previously reported by our lab [6]. Briefly, A β ₁₋₄₂ was dissolved in DMSO (10 mg/ml) and stored in aliquots at -20°C. To prepare A β oligomers (80 μ M), aliquots of the peptide (250 μ g in 25 μ l of DMSO) were added to 700 μ l of PBS (Gibco, USA) and vertically agitated (200 RPM at 37°C) for 90 min and stored at 4°C until use. A β -FAM was dissolved in DMSO (4 mg/ml) and immediately stored in aliquots at -20°C.

Transfection

Neuronal transfection was performed using magnetofection with the reagent Neuromag as described by the protocol provided by the manufacturer (Oz Biosciences, France). To decrease the levels of NMDARs, siRNAs for NR1 and NR2B were co-transfected with GFP (2 μ g total) to visualize the transfected neurons.

To increase the levels of NMDARs, HEK cells were transfected with the plasmids NR1, NR2B and GFP (2 μ g total) using lipofectamine 2000 (Invitrogen, USA) following the manufacturer's instructions. The plasmids and siRNAs used in this study were previously described [35, 36].

Electrophysiology

Electrophysiological recordings were carried out using the patch clamp technique as previously described [8, 37]. Briefly, culture media was changed for an external solution containing (in mM): 150 NaCl, 5.4 KCl, 2.0 CaCl₂, 1.0 MgCl₂, 10 glucose and 10 HEPES (pH 7.4). The internal solution consisted of (in mM): 120 KCl, 2.0 MgCl₂, 2 ATP-Na₂, 10 BAPTA, 0.5 GTP, 10 HEPES (pH 7.4). The holding potential was fixed at -60mV and currents were acquired using a Digidata 1200 board and the pClamp10 software (Axon Instruments, Inc.). Recording pipettes were pulled from borosilicate glass (WPI, Sarasota, FL) on a horizontal puller (Sutter Instruments, Novato, CA) having a resistance between 5 and 10 MΩ. Perforated recordings were obtained as previously described [6, 8]. Briefly, Aβ was added to the pipette internal solution and a 5mV pulse was used to monitor the perforation in cell attached configuration. For evoked current recordings, the experiments were performed at room temperature (20–25°C) using a membrane potential of -60mV. Data are given as mean±S.E.M. and were obtained from more than 5 experiments. Recordings were performed in the presence of 100 nM TTX (tetrodotoxin) to inhibit action potentials.

Immunocytochemistry

Experiments were performed as previously described [9]. The primary antibodies used for 16h were: anti-MAP2, 1:400 (Santa Cruz Biotechnology, CA, USA); and NR2B, 1:200 (Covance, USA). The secondary antibodies conjugated with FITC (ExMax/EmMax = 500/517 nm), Cy3 (ExMax/EmMax = 545/570 nm), or Cy5 (ExMax/EmMax = 649/670 nm) (1:400, Jackson ImmunoResearch Laboratories, USA) were incubated for 2h for fluorescent staining. Finally, samples were mounted in fluorescent mounting medium (DAKO, CA, USA) and images were obtained using a Nikon Eclipse confocal microscope (Nikon, Japan). The immunoreactivity of the proteins was quantified at primary processes with ImageJ software (NIH). Fluorescent signal was quantified as relative units (RU) using a region of interest (ROI).

Western blots

Equal amounts of proteins were separated on 10–12% SDS-PAGE gels as previously described [7, 8]. Protein bands were transferred onto nitrocellulose membranes, blocked with 5% milk, and incubated with the primary antibody anti-NR2B (1:1000; Covance, USA) or α -tubulin (Sigma, USA). Immunoreactive bands were detected with secondary antibodies conjugated with HRP 1:5000 (Santa Cruz Biotechnology, CA, USA) and visualized with ECL Plus Western Blotting Detection System (PerkinElmer, MA, USA).

Data analysis

Non-linear analysis was performed using Prism (Graph Pad). The analysis of the cluster size, number, and fluorescence intensity were performed using the ImageJ software package (NIH, USA) and using appropriate and similar intensity thresholds for cluster resolution. Membrane charge was analyzed by integrating the transient capacitive current after subtracting the pipette capacitance. The values are expressed as mean \pm SEM (standard error mean). Statistical differences were determined using Student's t test or ANOVA. The experiments were performed in triplicate.

Results

We wanted to evaluate the potential role of NMDARs in the mechanism of A β ₁₋₄₂ association and perforation of the plasma membrane. Therefore, we used experimental protocols that could either decrease or increase the levels of NMDARs in the cell membrane before performing the assays. First, we used a mild enzymatic proteolytic digestion using trypsin (0.00025%, 30 min), a serine protease widely used to remove membrane proteins [38, 39], thus decreasing the levels of NMDARs in the membrane of hippocampal neurons as detected by immunocytochemistry against the NR2B subunit of the receptor (Fig. 1A). More detailed analyses of the immunofluorescence data showed that treatment of hippocampal neurons with trypsin decreased the NMDAR puncta number in primary neuronal processes (Fig. 1B). For instance, control values were 12 \pm 1.2 punctas, and these values decreased to 6 \pm 1.10 punctas with the

treatment. This result is in agreement with western blot analyses that also showed a decrease of $60 \pm 12\%$ in NMDAR total levels after the treatment as compared to control (Fig. 1C,D). To evaluate if the treatment was actually reducing functional membrane NMDAR in the neurons treated with trypsin for 30 min, we performed electrophysiological recordings using patch clamp techniques. The neurons were stimulated with a large concentration of NMDA ($100 \mu\text{M}$) resulting in current amplitudes of several hundred pA. The results clearly showed a significant reduction in the amplitude of the NMDA-evoked current in trypsin-treated neurons (approximately 20%) as compared to the control condition (Fig. 1E). An internal control using the NMDAR antagonist, D-AP5 ($100 \mu\text{M}$), showed that the NMDA-evoked current was completely blocked by D-AP5, indicating that the evoked current was only mediated by NMDARs (Fig. 1E). As expected, the NMDA current density (pA/pF) was also significantly lowered in trypsin-treated neurons ($4.4 \pm 2 \text{ pA/pF}$) as compared to control cells ($20 \pm 2 \text{ pA/pF}$) (Fig. 1F). No differences were found in the values of membrane capacitance indicating that the treatment did not affect the size of the neurons (Fig. 1G). These data demonstrate that the use of a very low concentration of trypsin (0.00025%) is sufficient to decrease NMDAR levels in the neuronal membrane, making this experimental protocol a good tool for further assays which need decreased levels of this ion channel receptor.

The next step was to evaluate the degree of association of oligomeric $\text{A}\beta_{1-42}$ in control and trypsin treated neurons. Figure 2 shows a confocal micrograph of NR2B (red) and MAP2 (blue) together with the fluorescent signal of $\text{A}\beta$ (1 h incubation with $\text{A}\beta_{1-42}$ coupled to FAM, a green fluorescent tag) in control and trypsin-treated cells. The overall analysis of this data shows that after treating the hippocampal neurons with trypsin, the NR2B signal was reduced and that of $\text{A}\beta$ increased (Fig. 2A). The measurement of $\text{A}\beta$ clusters in primary neurites corroborated the increase in $\text{A}\beta$ -FAM association together with a decrease in NMDAR on trypsin-treated neurons (Fig. 2B). A more detailed analysis indicated that the $\text{A}\beta$ -associated clusters were much larger after the treatment (Fig. 2C). In fact, trypsin-treated neurons resulted in an increase in the size of $\text{A}\beta$ -FAM clusters together with the number of big clusters (Fig. 2C,D).

Furthermore, to characterize the role of NMDARs in A β association using more specific methodologies, we used siRNA for NMDAR subunits NR1 and NR2B to selectively decrease the NMDAR levels in these hippocampal neurons. Neuronal transfections for 48h with siRNAs also reduced the amplitude of NMDA-evoked currents (100 μ M) in patch clamp recordings (Fig. 3A). For instance, the data show that treatment with the siRNA decreased the amplitude of the NMDA current from 720 ± 96 to 347 ± 63 pA (Fig. 3B), resulting in a reduction of approximately 48%. After confirming that the siRNA reduced the expression of membrane NMDARs, we incubated the neurons for 1h with A β -FAM to assess the association to the neuronal membrane (Fig. 3C). The data obtained with immunocytochemistry showed that the siRNA-transfected neurons had an increase in A β -FAM association, incrementing the puncta number and the intensity of A β -FAM signal in primary processes (Fig. 3D,E), similar to the results obtained in trypsin-treated neurons.

Previous studies have shown that following A β association to the plasma membrane, a process of membrane damage begins that produces an increase in membrane current (perforation) and intracellular calcium levels which lead to a delayed synaptic failure [6–8, 40]. Thus, the increase in membrane association of A β_{1-42} to neurons depleted of membrane NMDARs could result in an increase in membrane damage. To determine if this was actually occurring, we performed electrophysiological experiments using perforated patch clamp recordings, as previously described [6, 8, 41] (see methods), and found that A β_{1-42} increased the peak and charge of the capacitive current after 15 minutes of application in control neurons (Fig. 4A). The effect of A β_{1-42} on the amplitude of the capacitive current, on the other hand, developed much quicker in neurons previously treated with trypsin to reduce NMDAR (Fig. 4A). The treatment, however, did not have any effect on the holding current. The time course of A β_{1-42} effects on membrane charge in control and after trypsin shows that the treatment caused an increase in the perforation onset (Fig. 4B). Data show that the approximate $t_{1/2}$ of A β effect on the perforation was 24min in control conditions and reduced to 17min with the treatment. Furthermore, the analysis of the time to acquire an open configuration (perforation) in control and trypsin-treated neurons showed that the effect of A β_{1-42} was concentration-dependent, with faster effects at higher concentrations (Fig. 4C). The

latter suggests that removal of membrane proteins, such as NMDARs, facilitates the membrane perforation induced by A β ₁₋₄₂.

Next, we examined if increasing NMDARs in the cell membrane could have the opposite effect, thus increasing the association of A β and the consequent perforation of the membrane. Thus, we overexpressed and electrophysiologically characterized the NMDAR subunits NR1 and NR2B in HEK cells (Fig. 5A), and glycine receptor as a control (Fig. 5B), evaluating the association of A β -FAM after 1 h incubation (Fig. 5C). Data showed that the A β -FAM association increased in the cells that overexpressed NMDARs, resulting in a reduced distance between the A β -FAM clusters (Control 0.55 ± 0.03 , NMDA-R 0.24 ± 0.01 pixels) and an increase in their size (Control 0.46 ± 0.05 , NMDA-R 0.97 ± 0.04 μ m) (Fig. 5B,C), which is similar to that observed when the levels of NMDARs were decreased (Figs. 2, 3). On the other hand, overexpression of another membrane protein that is associated to inhibitory transmission, the glycine receptor (GlyR), did not produce any change in A β -FAM association to the plasma membrane indicating that the effect was selective for NMDARs (Fig. 5). Thereafter, we evaluated the membrane perforation induced by A β ₁₋₄₂ in cells overexpressing NMDARs or GlyRs (Fig. 6A). The data obtained showed that NMDAR overexpression blocked the membrane perforation induced by A β ₁₋₄₂, while overexpression of GlyR was unable to affect the membrane charge transferred (Control 12.3 ± 5.3 , A β 155.3 ± 14.3 , NMDAR 25.4 ± 4.8 , GlyR α 1 135.9 ± 13.8 fC) (Fig. 6B). As a positive control for membrane perforation, we used a small peptide constructed with the native sequence of A β ₁₁₋₁₇ that includes the two histidine residues 13 and 14 (EVHHQKL) [42] which blocked the perforation of the membrane induced by A β (Fig. 6B) [6, 41, 42]. The latter suggests that NMDAR presence in the membrane of the cells increases A β association but interferes with the perforation induced by the peptide.

Discussion

The presence of a direct or indirect interaction between A β and NMDARs is still under discussion, however, the role of NMDARs in AD is becoming more recognized [28]. Previous studies, for example, are focused on the use of NMDAR antagonists, like memantine or (+) MK-

801 as AD treatments [29, 30]. In fact, memantine is one of the few FDA approved drugs for AD [43, 44].

In the present study, we characterized a largely unexplored area of the NMDAR relationship with A β regarding membrane association and subsequent perforation and that might have clinical implications. The original idea was to evaluate if the association of A β to the plasma membrane was affected by altering the levels of functional NMDARs, thus, cells were treated with trypsin to reduce receptor levels, or transfected with plasmids containing NMDAR subunits to increase the receptor levels. Interestingly, both treatments resulted in higher association of A β to the cells, suggesting a more complex role of NMDARs than expected. A simple way of explaining these results of A β association is what we call the "forest effect", where the membrane proteins can be likened to trees covering the ground (lipids) (Fig. 7). Thus, decreasing the level of NMDARs, suggested to be important for the interaction of A β with the neuronal membrane [21, 31], might be facilitating its association to lipids previously not accessible, resulting in enhanced clustering (Figs. 2 and 3). On the other hand, the increase in A β association after the overexpression of NMDARs might be due to enhanced direct or indirect interactions with these excitatory receptors. In agreement, it is now believed that A β has promiscuous membrane interactions associating to cellular prion, nicotinic receptors, A β PP, and lipids like GM1 and cholesterol, thus affecting A β clustering [9, 15, 17, 19–25]. Therefore, the increased A β association after overexpression of NMDARs could be explained by the formation of A β /NMDAR complexes [20, 31]. Interestingly, this effect was specific for NMDARs since overexpression of GlyRs did not affect A β association to the membrane. This differential effect might be relevant for the disease because GlyRs, unlike NMDARs, are inhibitory proteins that are mainly expressed in spinal cord neurons [45, 46], which are believed to be largely unaffected by the disease.

Interestingly, although decreasing or increasing NMDARs resulted in similar increases in A β association to the membrane (clustering), our data showed that the functional impact of altering the levels of NMDARs in the membrane was quite different. For instance, decreasing NMDARs resulted in an accelerated rate of brain membrane damage revealed as an increase in membrane current in the presence of A β . This is remarkable because it demonstrates differences on the

impact that A β association has on the plasma membrane. One plausible explanation is that the fine-tuning of A β interaction with membrane lipids and proteins affects its capacity to form amyloid pores. Thus, a membrane devoid of some types of proteins (i.e., NMDARs) might be more sensitive to A β -induced damage. It was reported that increasing protein levels in the membrane produced a reduction on its fluidity [47], thus our results might also be explained by a reduced level of membrane fluidity. Nevertheless, lipid rafts can also regulate A β association to the membrane [15, 17, 18]. For instance, cholesterol in lipid rafts can affect A β association and membrane damage by decreasing membrane fluidity as a consequence of reduced phospholipid movement in the bilayer [17, 48].

On the other hand, we found that the decrease in NMDAR levels by siRNAs or trypsin also increased A β association. In parallel, the perforation of the membrane was faster when compared to control cells (Fig. 5). We believe that by decreasing the levels of NMDARs, A β associates preferentially to membrane lipids facilitating the process of perforation [15, 16, 49]. For example, it is believed that GM1 mediates A β association, seeding, fibrillogenesis and membrane disruption [15, 49]. These results could very well explain why some people with high levels of A β in the brain do not show dementia and vice versa. In other words, some proteins might be buffering the levels of toxic A β [50].

In conclusion, our data support the role of NMDARs as an important mediator for A β association and damage/perforation in the plasma membrane, actively participating in the membrane toxicity induced by A β . These results could explain the lack of correlation between brain A β burden and clinically observed dementia.

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Figures and Tables

Fig. 1. Treatment with trypsin decreased NMDARs in hippocampal neurons. A) Microphotograph shows NMDAR (NR2B, red) in neuronal primary processes of cultures treated without or with trypsin (0.00025%, 30 min). B) Quantification of NMDAR puncta number shows a decrease in trypsin-treated neurons (0.00025%, 30 min) versus control (not treated). C) Western blot showing the levels of NR2B in control and trypsin-treated neurons (0.00025%, 30 min). α -tubulin was used as a loading control. D) Quantification of NR2B levels from the western blot in control and treated neurons. E) Representative evoked currents using NMDA (100 μ M) and NMDA plus D-AP5 (100 μ M) in control neurons and pre-treatment with trypsin 0.00025% for 30 min. Black bar represents the time of perfusion. F) Plot of current density (pA/pF) in control and trypsin pre-treated neurons showing the current decrease in trypsin-treated neurons. G) The graph shows the capacitance (pF) in control and trypsin-treated cells. ** $p < 0.01$; *** $p < 0.001$.

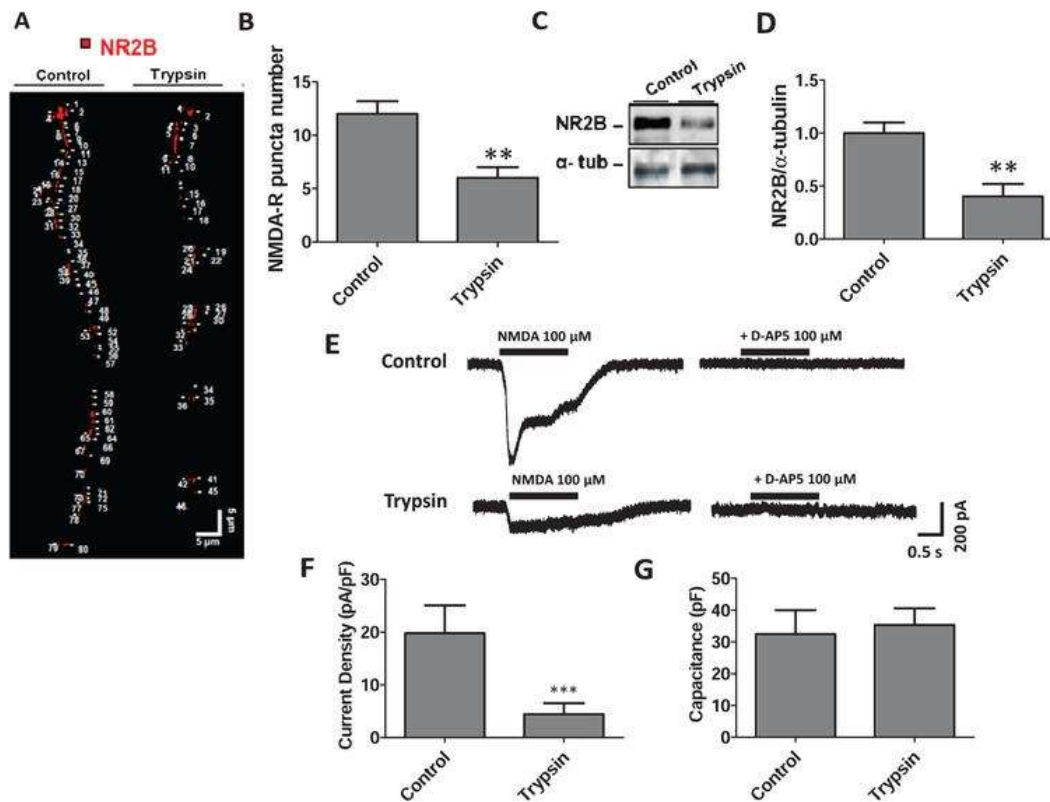


Fig. 2. Trypsin treatment decreased NMDARs and increased A β association in hippocampal neurons. A) Immunofluorescence microphotograph showing the association of A β -FAM (1 μ M, 1 h) to hippocampal neurons pre-treated with or without trypsin (0.00025%, 30 min). NMDA receptor is shown in red (NR2B subunit), A β -FAM in green and MAP2 in blue. Scale bar represents 20 μ m. B) Representative images of neuronal primary processes (10 μ m) showing the levels of NMDAR (red) and the association of A β -FAM (green, 1 h, 1 μ M) in control and trypsin pre-treated (0.00025%, 30 min) neurons. The panel at the right displays a zoom of the dendrite. C) Plot shows the quantification of A β -FAM cluster size (μ m). D) Graph illustrates the relationship between the number and size (nm) of A β -FAM clusters in control and treated neurons. ** $p < 0.01$.

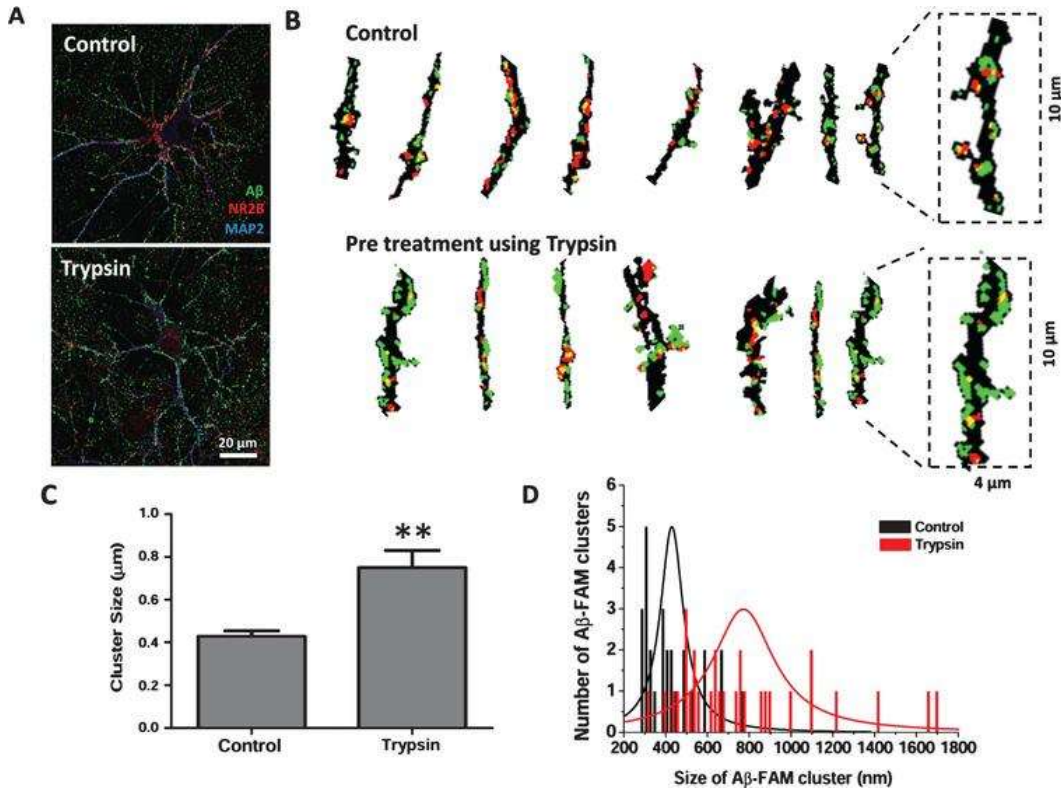


Fig. 3. NMDARs affect A β association in hippocampal neurons. A) Representative traces of NMDA-evoked currents in control and after transfection with siRNAs geared towards the NMDAR (NR1 and NR2B subunits). The black bar represents the time of NMDA perfusion (100 μ M). B) Plot showing the decrease in the amplitude of NMDA-evoked currents for the siRNA-transfected neurons versus control. C) Immunofluorescence showing A β -FAM (green) association (1 μ M, 1 h) to control and siRNA transfected neurons. MAP2 stained the neurons (blue) and mCherry was used as a control for transfection (red). The white bottom bar represents 20 μ m of length. D, E) Plots show the A β -FAM puncta number and fluorescence intensity in primary processes (20 μ m) for control and transfected neurons. * p < 0.05, ** p < 0.01.

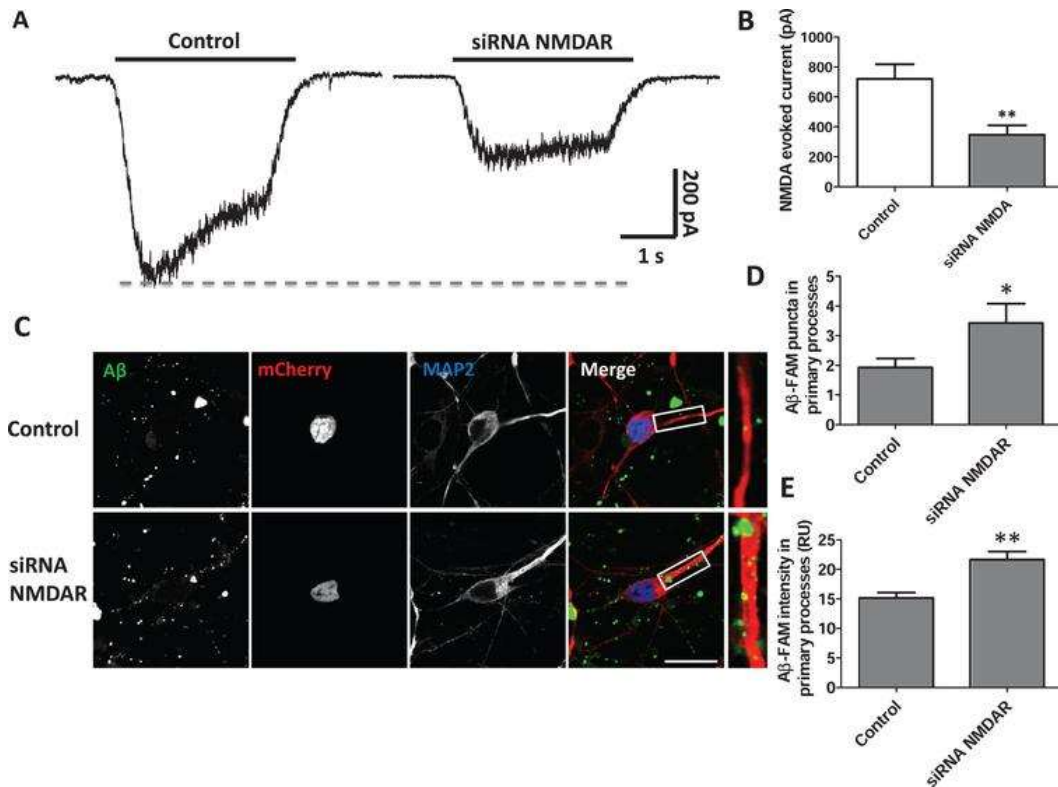


Fig. 4. Trypsin treatment decreased the time to perforated configuration induced by A β . A) Representative capacitive currents (5mV) in the perforated configuration using A β (1 μ M) in the pipette in HEK cells. B) Plot showing the charge transferred through the membrane of the cell in a time dependent manner, showing that A β perforates the membrane faster in trypsin pre-treated cells (0.00025%, 30 min) than in control cells without any treatments. Arrow heads (red) indicate the times of the recordings shown in panel A. C) Graph showing the time needed by A β to acquire a perforation configuration. The trypsin pre-treated cells had a faster perforation time. * $p < 0.05$, ** $p < 0.01$.

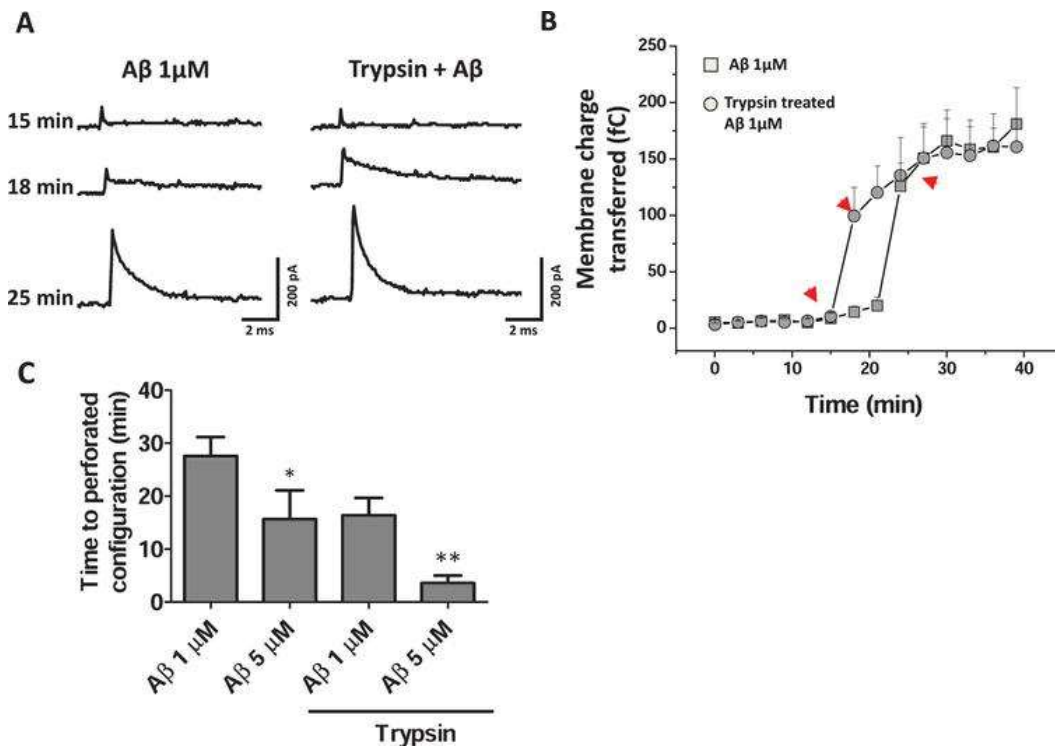


Fig. 5. NMDAR overexpression increased A β -FAM clustering in HEK cells. A) The trace shows NMDA-evoked current in HEK cells transfected with NR1 and NR2B. B) The trace shows a glycine evoked current in HEK cells transfected with the alpha subunit of the glycine receptor (GlyR α 1). C) Microphotograph showing A β -FAM association (1 μ M, 1 h) to HEK cells overexpressing NMDAR (NR1/NR2B), GlyR α 1 and RFP as a control for transfection. D) Plot showing the A β -FAM cluster inter-distance. E) Graph showing the increase in A β -FAM cluster size in cells that overexpress NMDAR. ** $p < 0.01$.

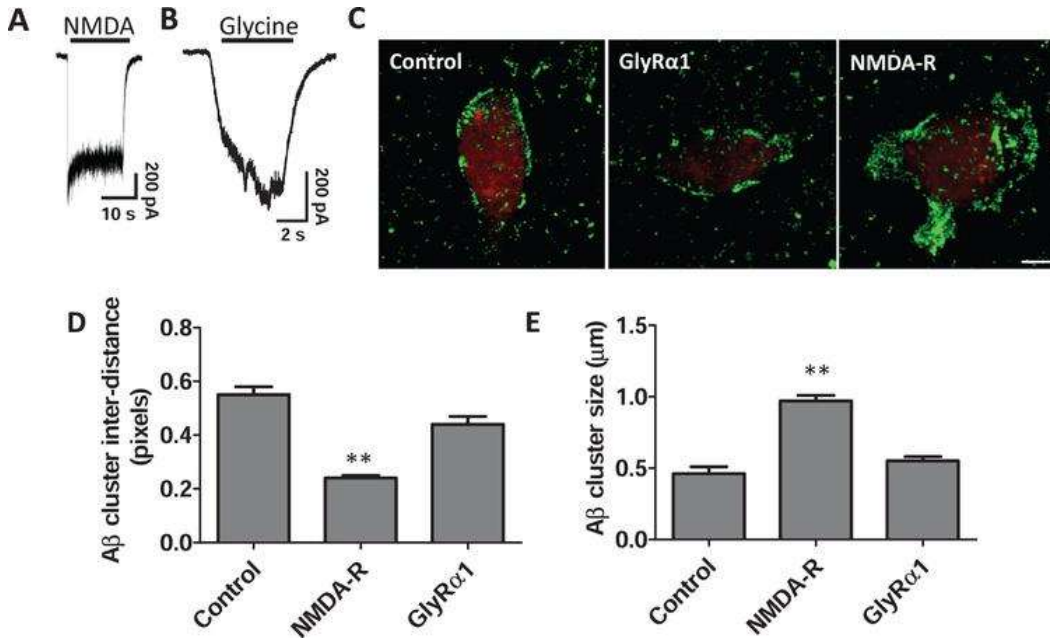


Fig. 6. NMDAR overexpression inhibited the A β -induced membrane perforation. A) Scheme representing the perforated patch clamp configuration used in the experiment. A β is added to the patch pipette with the internal solution and the cell-attached configuration is acquired. The recorded cells were previously transfected with GFP, NMDAR or GlyR α 1. B) Plot showing the membrane charge transferred (fC) in control HEK cells and HEK cells transfected with GFP, NMDAR or GlyR α 1 after 20 min in the presence of A β (1 μ M). The cells that overexpressed NMDARs were resistant to A β . Na7, a peptide that blocks the amyloid pore, was used as a control for the A β perforation. ** $p < 0.01$, *** $p < 0.001$.

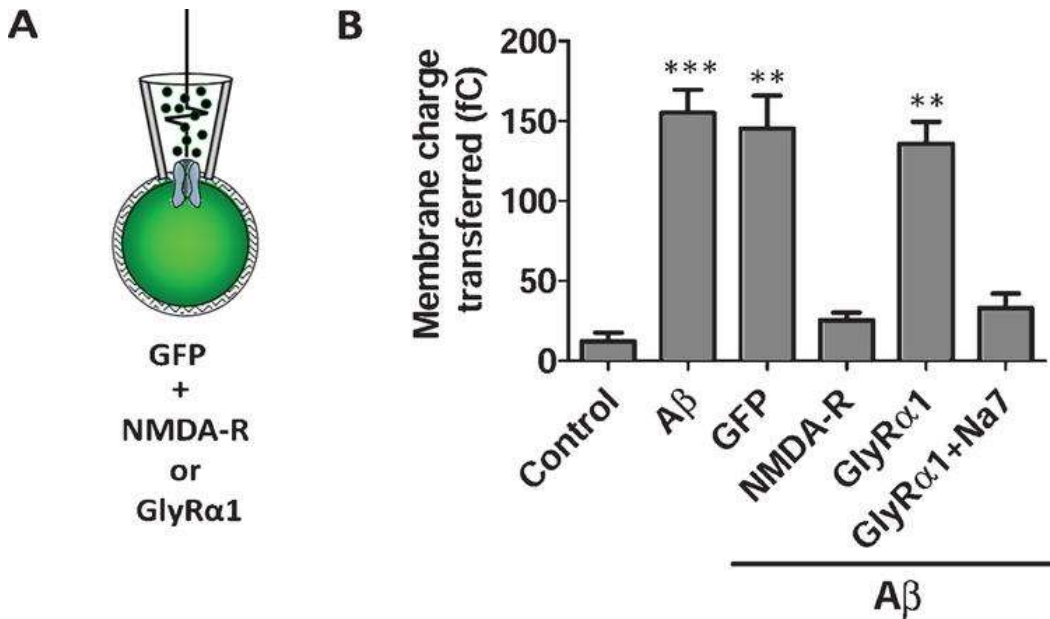
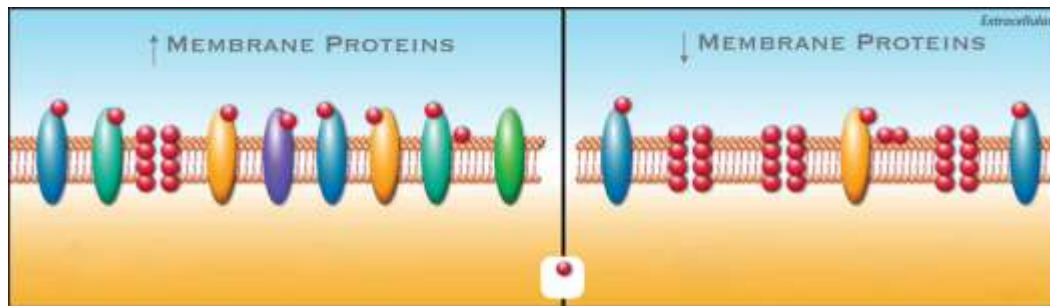


Fig. 7. "Forest effect" of membrane proteins involved in A β association and membrane perforation. A) The increase in membrane protein levels, such as NMDAR, augments the association of A β (red circles) to these proteins, decreasing the association of A β to membrane lipids and therefore the perforation induced by A β . B) The decrease in membrane proteins, i.e., NMDAR, results in an increase in A β association to the lipids of the plasma membrane, thus increasing the perforation induced by A β .



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