



# Molecular Modulation of Human $\alpha 7$ Nicotinic Receptor by Amyloid- $\beta$ Peptides

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Amyloid  $\beta$  peptide ( $A\beta$ ) is a key player in the development of Alzheimer's disease (AD). It is the primary component of senile plaques in AD patients and is also found in soluble forms. Cholinergic activity mediated by  $\alpha 7$  nicotinic receptors has been shown to be affected by  $A\beta$  soluble forms. To shed light into the molecular mechanism of this effect, we explored the direct actions of oligomeric  $A\beta_{1-40}$  and  $A\beta_{1-42}$  on human  $\alpha 7$  by fluorescence spectroscopy and single-channel recordings. Fluorescence measurements using the conformational sensitive probe crystal violet (CrV) revealed that in the presence of  $A\beta$   $\alpha 7$  undergoes concentration-dependent conformational changes. Exposure of  $\alpha 7$  to 100 pM  $A\beta$  changes CrV  $K_D$  towards that of the desensitized state. However,  $\alpha 7$  is still reactive to high carbamylcholine (Carb) concentrations. These observations are compatible with the induction of active/desensitized states as well as of a novel conformational state in the presence of both  $A\beta$  and Carb. At 100 nM  $A\beta$ ,  $\alpha 7$  adopts a resting-state-like structure which does not respond to Carb, suggesting stabilization of  $\alpha 7$  in a blocked state. In real time, we found that  $A\beta$  is capable of eliciting  $\alpha 7$  channel activity either in the absence or presence of the positive allosteric modulator (PAM) PNU-120596. Activation by  $A\beta$  is favored at picomolar or low nanomolar concentrations and is not detected at micromolar concentrations. At high  $A\beta$  concentrations, the mean duration of activation episodes elicited by ACh in the presence of PNU-120596 is significantly reduced, an effect compatible with slow open-channel block. We conclude that  $A\beta$  directly affects  $\alpha 7$  function by acting as an agonist and a negative modulator. Whereas the capability of low concentrations of  $A\beta$  to activate  $\alpha 7$  could be beneficial, the reduced  $\alpha 7$  activity in the presence of higher  $A\beta$  concentrations or its long exposure may contribute to the cholinergic signaling deficit and may be involved in the initiation and development of AD.

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**Abbreviations:** ACh, acetylcholine; Carb, carbamylcholine;  $A\beta_{1-42}$ , amyloid- $\beta_{1-42}$  peptide;  $A\beta_{1-40}$ , amyloid- $\beta_{1-40}$  peptide; nAChR, nicotinic acetylcholine receptor; CrV, crystal violet; NS-1738, N-(5-Chloro-2-hydroxyphenyl)-N'-[2-chloro-5-(trifluoromethyl) phenyl]urea; PNU-120596, [N-(5-Chloro-2,4-dimethoxyphenyl)-N'-(5-methyl-3-isoxazolyl)-urea].

## INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by loss of memory, multiple cognitive impairments and changes in personality and behavior. Memory impairment in AD is associated with neuronal degeneration as well as synaptic damage. Although AD is a multifactorial disease, accumulation of amyloid- $\beta$  peptides (A $\beta$ ) is one of the major pathological factors. Accumulation phase starts with low molecular weight fractions of A $\beta$  (monomers, dimers, or trimers) and continues with larger oligomers or insoluble amyloid fibrils (Sadigh-Eteghad et al., 2014). Although plaques remain the principal identifiers and predictors of Alzheimer's disease, a clear paradigm shift has occurred that emphasizes the primacy of A $\beta$  oligomers in disease causation (Lacor et al., 2004; Shankar et al., 2008; Hayden and Teplow, 2013; Collins-Praino et al., 2014). Despite all research efforts, there are still many unsolved aspects regarding the molecular mechanisms underlying A $\beta$  pathogenic actions. One of these mechanisms involves the interaction of A $\beta$  with synaptic receptors, which consequently emerge as novel druggable sites to restore cognitive functions in AD patients (Kandimalla and Reddy, 2017).

Cholinergic neurons with a pivotal role in learning and memory are mainly involved in the pathogenesis of AD. Indeed, inhibitors of acetylcholinesterase (AChE), which by decreasing ACh breakdown enhance cholinergic neurotransmission, are to date one of the main specific therapeutic drugs, although their efficacy is limited (Kandimalla and Reddy, 2017).

The  $\alpha$ 7 nicotinic acetylcholine receptor (nAChR) has been shown to be associated with AD.  $\alpha$ 7 is highly expressed in hippocampus, cortex and several subcortical limbic regions and is involved in cognition, sensory processing information, attention, working memory, and reward pathways (Lendvai et al., 2013). Reduction of  $\alpha$ 7 in brain, particularly in the hippocampus, has been reported in AD patients (Buckingham et al., 2009; Dineley et al., 2015).

$\alpha$ 7 is a pentameric ligand-gated ion channel that responds to ACh by opening an intrinsic channel permeable to cations that triggers rapid membrane depolarization and calcium influx (Wonnacott, 2014). However,  $\alpha$ 7 also acts as a metabotropic receptor and triggers several signal transduction pathways as well as the release of calcium from intracellular stores (Kabbani et al., 2013; Egea et al., 2015; Guan et al., 2015; Corradi and Bouzat, 2016; Bouzat et al., 2018). This metabotropic activity has been associated to synaptic plasticity and neuroprotection, including against A $\beta$  damage (Buckingham et al., 2009; Inestrosa et al., 2013; Jin et al., 2015).

Enhancement of  $\alpha$ 7 activity is emerging as a therapeutic strategy for cognitive impairment in AD. Positive allosteric modulators (PAMs) are the most promising therapeutic compounds because they maintain the temporal and spatial characteristics of endogenous activation, are more selective than agonists, and reduce tolerance due to desensitization (Chatzidakis and Millar, 2015; Terry et al., 2015; Corradi and Bouzat, 2016; Echeverria et al., 2016; Yang et al., 2017; Bouzat et al., 2018). Based on their effects on macroscopic currents, PAMs have been classified as type I PAMs, that mainly enhance agonist-induced

peak currents, and type II PAMs, that enhance agonist-elicited currents and also decrease desensitization and recover receptors from desensitized states (Bertrand and Gopalakrishnan, 2007; Grønlien et al., 2007; Andersen et al., 2016).

Both  $\alpha$ 7 agonist- and antagonist-like actions of A $\beta$  have been described in different cells and tissues (Wu et al., 2004; Khan et al., 2010; Li et al., 2011; Parri et al., 2011; Sadigh-Eteghad et al., 2014; Liu et al., 2015; Yan et al., 2015). Studies have been focused mainly on evaluation of the effects of A $\beta$  on  $\alpha$ 7 metabotropic activity, which includes signaling pathways, such as ERK/MAPK, PI3K/AKT, JAK-2/STAT-3 and intracellular calcium mobilization. The acute effects of A $\beta$  on  $\alpha$ 7 electrical activity have been explored at the macroscopic current level. Despite problems of comparability and some dissimilar results, probably due to variations on the aggregation state and concentration of amyloid peptides (see Buckingham et al., 2009), the consensus indicates that low concentrations of A $\beta$  (picomolar) activate  $\alpha$ 7 whereas higher concentrations lead to current inhibition (Liu et al., 2001; Dineley et al., 2002; Grassi et al., 2003; Pym et al., 2005; Parri et al., 2011).

To shed light into the molecular mechanism of the direct action of A $\beta$  on  $\alpha$ 7, we expressed human  $\alpha$ 7 on mammalian cells and evaluated functional effects by fluorescence spectroscopic measurements and electrophysiological recordings. We used A $\beta$  preparations enriched in oligomeric forms since these species were shown to be involved in cognitive impairment, inhibition of long-term potentiation, memory loss and  $\alpha$ 7 modulation (Walsh et al., 2002; Wang et al., 2002; Cleary et al., 2005; Parri et al., 2011). By taking advantage of the potential of single-channel recordings in providing information unattainable by macroscopic measurements, we deciphered the direct molecular effects of oligomeric A $\beta$  as activator and inhibitor of  $\alpha$ 7 channels. By using a fluorescent conformational probe, we revealed that A $\beta$  elicits different concentration-dependent conformational changes and induces novel conformational states.

## MATERIALS AND METHODS

### Drugs

Acetylcholine (ACh) and human amyloid- $\beta$ 1–42 (A $\beta$ <sub>1–42</sub>) were purchased from Sigma-Aldrich (St. Louis, MO, USA); NS-1738 N-(5-Chloro-2-hydroxyphenyl)-N'-[2-chloro-5-(trifluoromethyl) phenyl]urea, PNU-120596 [N-(5-Chloro-2,4-dimethoxyphenyl)-N'-(5-methyl-3-isoxazolyl)-urea] and rat amyloid- $\beta$ 1–40 (A $\beta$ <sub>1–40</sub>) were obtained from Tocris Biosciences (Bristol, UK).

### Receptor Expression

BOSC-23 cells, derived from HEK 293 cells (Pear et al., 1993), were transfected by calcium phosphate procedure with human  $\alpha$ 7 cDNA subcloned in pRBG4 vector (Bouzat et al., 1994). Plasmids harboring cDNAs of the  $\alpha$ 7 chaperone proteins Ric-3 and NACHO were incorporated to favor  $\alpha$ 7 expression (Bouzat et al., 2008; Andersen et al., 2016; Nielsen et al., 2018). All transfections were carried out for about 12–18 h in Dulbecco's Modified Eagle Medium (DMEM) with 10%

fetal bovine serum and terminated by exchanging the medium. Cells were used for single-channel recordings 2–4 days after transfection. To facilitate identification of transfected cells, a separate plasmid encoding green fluorescent protein was included in all transfections.

## Amyloid- $\beta$ Peptide Preparations

A $\beta$ <sub>1–40</sub> or A $\beta$ <sub>1–42</sub> were resuspended in dimethyl sulfoxide at a concentration of 10 mg/ml (peptide stock solution) and stored in aliquots at  $-80^{\circ}\text{C}$ . A $\beta$  oligomers were prepared immediately prior to use according to previously published methods (Uranga et al., 2016; Pascual et al., 2017). Briefly, aliquots of peptide stock (10  $\mu\text{l}$ ) were added to 280  $\mu\text{l}$  of phosphate buffered saline (PBS; pH 7.4) and stirred continuously (300 rpm) for 120 min and stored at  $4^{\circ}\text{C}$  until use.

Transmission electron microscopy (TEM) studies were carried out according to previously published methods with slight modifications (Uranga et al., 2016; Pascual et al., 2017). Briefly, 10  $\mu\text{l}$  media containing the peptide was placed on a carbon-coated grid and incubated for 60 s. Ten microliters of 0.5% glutaraldehyde was added to the grid followed by incubation for an additional 60 s. The grid was then washed with drops of water and dried. Finally, the grid was stained for 2 min with 2% uranyl acetate and air dried. The grid was subsequently examined under a Jeol 100 Cx II electron microscope (Uranga et al., 2016). TEM photomicrographs showed that A $\beta$  preparations included spheroidal structures individually or in small groups (**Supplementary Figure S1**). These ultrastructural forms were compatible with a heterogeneous array of oligomers, thus discarding the presence of fibrils (Uranga et al., 2016; Pascual et al., 2017).

## Single-Channel Recordings

Single-channel recordings were obtained in the cell-attached patch configuration. The bath and pipette solutions contained 142 mM KCl, 5.4 mM NaCl, 1.8 mM CaCl<sub>2</sub>, 1.7 mM MgCl<sub>2</sub> and 10 mM HEPES (pH 7.4). For potentiation, 1  $\mu\text{M}$  PNU-120596 or 10  $\mu\text{M}$  NS-1738 were added to the pipette solution together with ACh.

Single-channel currents were digitized at 5–10  $\mu\text{s}$  intervals, low-pass filtered at a cut-off frequency of 10 kHz using an Axopatch 200B patch-clamp amplifier (Molecular Devices Corp., CA, USA). Single-channel events were idealized by the half amplitude threshold criterion using the program QuB 2.0.0.28 (Qin et al., 1996, 1997; State University of New York at Buffalo) with a digital low-pass filter at 9 kHz. A filter of 3 kHz was used in recordings with PNU-120596 to facilitate the analysis. The open and closed time histograms obtained from idealization were fitted by the maximum interval likelihood (MIL) function in QuB (Qin et al., 1996, 1997), with a dead time of 0.1 ms. This analysis was performed by sequentially adding an open and/or closed state to a starting C  $\leftrightarrow$  O model in order to properly fit the corresponding histograms (Fabiani et al., 2018; Lasala et al., 2018). Final models contained five-six closed states and three-four open states for  $\alpha$ 7 in the presence of ACh plus PNU-120596, five-six closed states and three open states for  $\alpha$ 7 in

the presence of ACh plus NS-1738, or three closed states and one-two open states for  $\alpha$ 7 in the presence of ACh and absence of PAMs.

Clusters were identified as a series of closely separated openings preceded and followed by closings longer than a critical duration. Different critical closed times were calculated by MIL between each closed component. Critical times between the third and fourth closed components for  $\alpha$ 7 in the presence of PNU-120596 ( $\sim$ 30–60 ms) or NS-1738 ( $\sim$ 2–8 ms) were selected in QuB to chop the idealized data and create a sub-data set that only contained clusters to define mean cluster duration.

## Fluorimetric Measurements

Fluorimetric measurements were performed in a SLM model 4800 fluorimeter (SLM Instruments, Urbana, IL, USA) using a vertically polarized light beam from an Hannoveria 200-W mercury/xenon arc obtained with a Glan-Thompson polarizer (4 nm excitation and emission slits).

Crystal violet (CrV) was used as a probe to detect conformational changes (Lurtz and Pedersen, 1999; Sun et al., 2017; Fabiani et al., 2018). nAChR-rich membranes were prepared from *Torpedo californica* electric tissue as described previously (Fernández Nievas et al., 2008; Perillo et al., 2012; Fabiani et al., 2018). CrV experiments using *Torpedo* membranes or  $\alpha$ 7-expressing cells were conducted as described previously for the *Torpedo* nAChR (Fernández Nievas et al., 2008; Perillo et al., 2012; Fabiani et al., 2018). *Torpedo* membranes or BOSC-23 cells expressing  $\alpha$ 7 were resuspended on phosphate saline buffer with a protein concentration of 100  $\mu\text{g}/\text{ml}$  or up to an absorbance value of  $\sim$ 0.5 at 280 nM measured on a Jasco V-630 spectrophotometer (JASCO Deutschland GmbH), respectively. The suspended cells or membranes were incubated with A $\beta$  oligomers for 20 min. For measurements conducted in the desensitized state, the membrane or cell suspensions were additionally incubated for 15 min with 1 mM or 20 mM carbamylcholine (Carb), respectively. The suspensions were subsequently titrated with increasing concentrations of CrV (in water). After each addition of CrV, the samples were incubated for 15 min before obtaining the fluorescence emission spectra. CrV was excited at 600 nm, and the fluorescence emission spectra were collected from 605 to 700 nm. Before the first addition of CrV, a background fluorescence emission spectrum was obtained for each sample. The spectrum was then subtracted from the emission spectra obtained in the presence of CrV and the maximum intensity (at 623–625 nm) was measured. To determine the CrV dissociation constant ( $K_D$ ), the value of the CrV maximum fluorescence emission was plotted as a function of the logarithmic CrV concentration ( $M$ ). The resulting sigmoid curve was fitted by the Boltzmann function and the  $K_D$  was calculated using the program Origin 7.0 (OriginLab Corporation).

## Statistical Analysis

Intergroup comparisons were carried out using one-way analyses of variance (ANOVA), Dunnett's multiple comparisons

test (Graphpad PRISM software). The values represent the average  $\pm$  SD of the total number of samples indicated. Statistically significance difference was established at  $p$ -values  $<0.05$ .

## RESULTS

### Amyloid $\beta$ Oligomers Induce Conformational Changes in $\alpha$ 7

To examine the potential of oligomeric A $\beta$  peptides to directly affect  $\alpha$ 7, we first used the conformational-sensitive probe CrV and measured its affinity for the receptor before and after exposure to A $\beta$  by fluorescence spectroscopy.

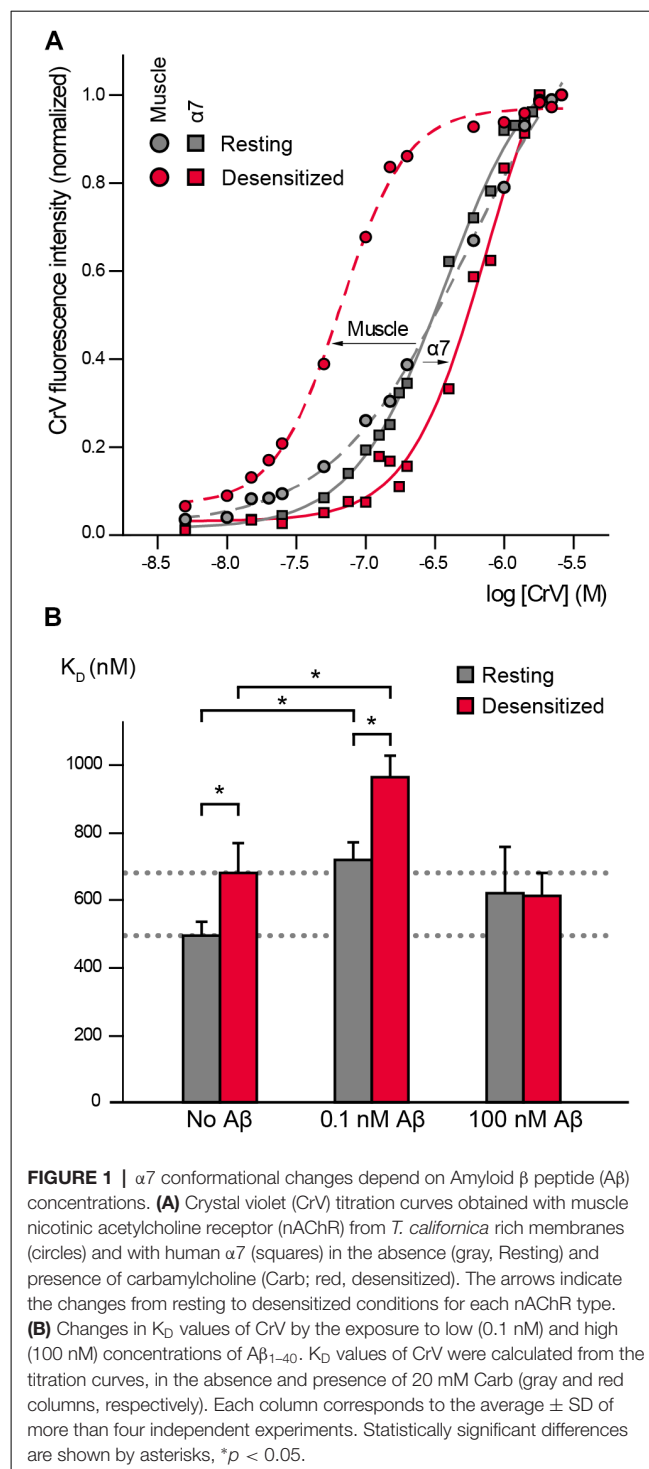
CrV has been extensively used for studies in the *Torpedo* muscle nAChR where it shows low affinity for the resting state ( $K_D$  values  $\sim$ 400 nM, Fernández Nievas et al., 2008; Perillo et al., 2012) and high affinity for the desensitized state ( $K_D$  values  $\sim$ 60 nM; Fernández Nievas et al., 2008; Perillo et al., 2012; Fabiani et al., 2018; **Figure 1A**).

We first determined if CrV can be used as a conformational probe for the human  $\alpha$ 7 receptor. Suspensions of BOSC-23 cells expressing  $\alpha$ 7 were titrated with increasing concentrations of CrV. Saturable CrV binding yielded a  $K_D$  value of  $492 \pm 41$  nM for resting  $\alpha$ 7 receptors, indicating an affinity similar to that for the muscle nAChR in the resting state. Interestingly, measurements in the desensitized state, which was induced by 20-min preincubation with Carb, yielded a  $K_D$  value of  $680 \pm 89$  nM for CrV binding (**Figures 1A,B**). The maximal fluorescence intensities of the samples were similar at resting and desensitized conditions, thus discarding that the lower affinity for the desensitized state corresponds to reduced binding of the probe in the latter state. These results indicate that CrV saturates its binding sites at both conditions with different affinities. Thus, opposite to its binding affinity profile for the muscle nAChR, CrV shows higher affinity for resting than for desensitized states in  $\alpha$ 7.

We next determined the effects of low (0.1 nM) and high (100 nM) A $\beta_{1-40}$  concentrations on  $\alpha$ 7 conformation by measuring CrV  $K_D$  values to resting and desensitized states (**Figure 1B**).

Exposure of  $\alpha$ 7 in the resting state (in the absence of Carb) to 0.1 nM A $\beta_{1-40}$  increased the CrV  $K_D$  towards that of the desensitized state, indicating that amyloid peptides induce conformational changes in  $\alpha$ 7. Interestingly, subsequent addition of Carb induced a further displacement to higher  $K_D$  values, suggesting that the agonist can lead to further conformational changes (**Figure 1B**).  $K_D$  values for desensitized conditions were statistically significantly different in the absence and presence of A $\beta$  (**Figure 1B**). Similar results were obtained with 0.1 nM A $\beta_{1-42}$ .

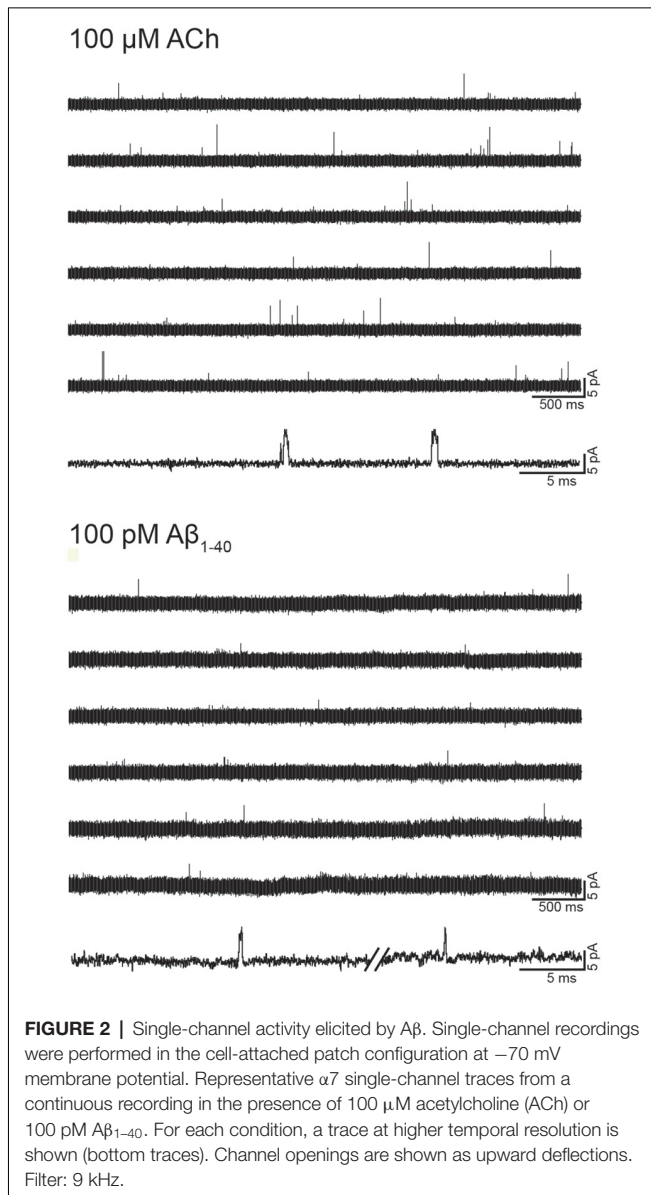
On the other hand, incubation of resting  $\alpha$ 7 receptors with 1,000-fold higher A $\beta_{1-40}$  concentration (100 nM) did not induce statistically significant changes in the CrV  $K_D$  value (**Figure 1B**). Furthermore, the  $K_D$  value remained constant even after addition of 20 mM Carb, indicating that  $\alpha$ 7 was not reactive to the agonist. Similar results were obtained with high (100 nM) A $\beta_{1-42}$ .



**FIGURE 1** |  $\alpha$ 7 conformational changes depend on Amyloid  $\beta$  peptide (A $\beta$ ) concentrations. **(A)** Crystal violet (CrV) titration curves obtained with muscle nicotinic acetylcholine receptor (nAChR) from *T. californica* rich membranes (circles) and with human  $\alpha$ 7 (squares) in the absence (gray, Resting) and presence of carbamylcholine (Carb; red, desensitized). The arrows indicate the changes from resting to desensitized conditions for each nAChR type. **(B)** Changes in  $K_D$  values of CrV by the exposure to low (0.1 nM) and high (100 nM) concentrations of A $\beta_{1-40}$ .  $K_D$  values of CrV were calculated from the titration curves, in the absence and presence of 20 mM Carb (gray and red columns, respectively). Each column corresponds to the average  $\pm$  SD of more than four independent experiments. Statistically significant differences are shown by asterisks, \* $p < 0.05$ .

### Oligomeric Amyloid- $\beta$ Peptides at Low Concentrations Trigger Human $\alpha$ 7 Channel Opening

Once established that oligomeric A $\beta$  peptides induce conformational changes that are sensitive to concentration, we took advantage of the potential of single-channel recordings to reveal the mechanistic basis of this modulation.



To first determine if A $\beta$  can activate human  $\alpha$ 7, we examined single-channel activity from BOSC-23 cells expressing the receptor (Figure 2). In the presence of  $100 \mu\text{M}$  ACh,  $\alpha$ 7 exhibits single brief openings flanked by long closings, or less often, several openings in quick succession, which are called bursts (Bouzat et al., 2008; Andersen et al., 2016). The mean open duration was  $0.36 \pm 0.07 \text{ ms}$  ( $n = 3$ ) and the mean burst duration was  $0.77 \pm 0.21 \text{ ms}$  ( $n = 3$ ; Figure 2). No  $\alpha$ 7 channel activity was detected in the absence of agonist. However,  $100 \text{ pM}$  A $\beta_{1-40}$  elicited the typical  $\alpha$ 7 channel openings (Figure 2). The number of active patches was lower than in the presence of ACh (28% transfected cells showed channel activity). Channel activity was not detected if A $\beta_{1-40}$  was increased to  $10 \text{ nM}$  and  $100 \text{ nM}$  ( $n = 16$  patches of different cells and transfections), indicating that activation is favored at low concentrations. These experiments were performed in parallel with recordings with

ACh as the agonist to discard that the lack of channel activity was due to the lack of functional expression.

We next performed recordings in the presence of the type II PAM PNU-120596, which increases the probability of agonist-elicited channel opening and, consequently, favors the detection of infrequent opening events (daCosta et al., 2011; Andersen et al., 2016). By itself, PNU-120596 cannot elicit channel activation (Hurst et al., 2005). In the presence of  $1 \mu\text{M}$  PNU-120596,  $\alpha$ 7 channel activity elicited by ACh ( $100 \mu\text{M}$ ) appears in long activation periods of high frequency, named clusters, whose mean duration is about 1–3 s (Figure 3, Table 1; Andersen et al., 2016).

In the absence of ACh, A $\beta_{1-40}$  or A $\beta_{1-42}$  at a low concentration ( $100 \text{ pM}$ ) elicited clusters of PNU-120596-potentiated  $\alpha$ 7 channels (Figure 3). Again, the proportion of active patches was lower than in the presence of ACh. Whereas almost all patches (>90%) showed channel activity with ACh, the percentage of active patches was reduced in the presence of A $\beta_{1-40}$  or A $\beta_{1-42}$  as agonists (Table 1). Moreover, as A $\beta$  concentration increased, the number of patches showing  $\alpha$ 7 activity decreased. As shown in Table 1, at  $100 \text{ pM}$  43% and 78% of the patches showed clusters elicited by A $\beta_{1-40}$  or A $\beta_{1-42}$ , respectively, but no channel activity was detected at  $100 \text{ nM}$ . Thus, also in the presence of a PAM, activation by A $\beta$  is favored at picomolar or low nanomolar concentrations.

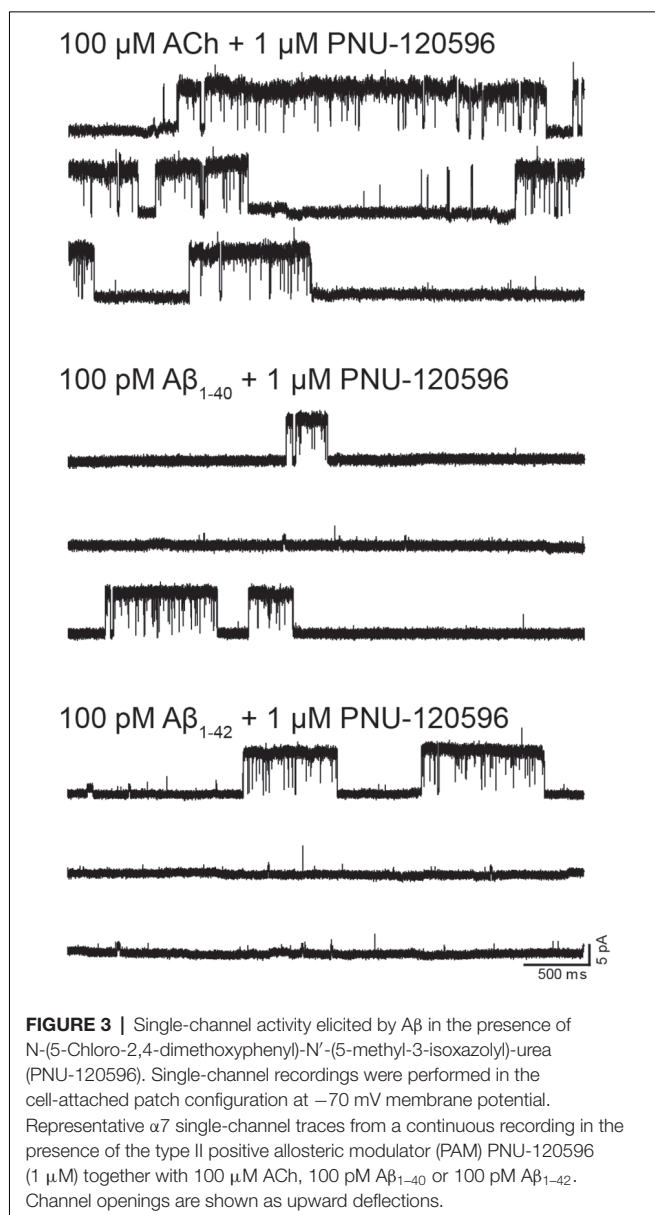
Although the frequency of clusters is usually variable among patches from different cell transfections, it was systematically lower in the presence of A $\beta$  with respect to ACh as illustrated in typical recordings shown in Figure 3. For a better comparison, recordings with ACh or A $\beta$  were performed in the same batch of transfected cells.

In the presence of ACh and PNU-120596, each cluster is composed of two or three bursts that contain long-duration openings separated by brief closings (daCosta et al., 2011; Andersen et al., 2016). Clusters elicited by A $\beta$  in the presence of PNU-120596 showed the typical architecture observed with ACh (Figure 3). The analysis showed no statistically significant differences in the mean cluster duration at all tested

**TABLE 1 |** Activation of  $\alpha$ 7 by Amyloid  $\beta$  peptide (A $\beta$ ) in the presence of N-(5-Chloro-2,4-dimethoxyphenyl)-N'-(5-methyl-3-isoxazolyl)-urea (PNU)-120596.

[ACh] ( $\mu\text{M}$ )	A $\beta$	[A $\beta$ ] (nM)	% active patches (n)	Mean cluster duration (ms)
100	No	0	100 (6)	$2263 \pm 990$
0	A $\beta_{1-40}$	0.1	42.9 (7)	$762 \pm 316$
0		1	22.2 (18)	$2016 \pm 1364$
0		10	30.0 (10)	$5229 \pm 1392^*$
0	A $\beta_{1-42}$	100	0 (9)	Nd
0		0.1	77.8 (9)	$1401 \pm 805$
0		1	46.2 (13)	$1208 \pm 946$
0		10	33.3 (9)	$1751 \pm 766$
0		100	0 (6)	Nd

Single-channel currents from cells expressing human  $\alpha$ 7 were recorded in the presence of  $1 \mu\text{M}$  PNU-120596 plus  $100 \mu\text{M}$  acetylcholine (ACh) or A $\beta$  at the indicated concentrations. The mean cluster duration was obtained from the corresponding histograms. Nd: not detected. Statistical comparisons were performed against the corresponding control condition using analyses of variance (ANOVA; Dunnett's multiple comparisons test). \* $p < 0.05$ .



concentrations, except for  $10 \text{ nM}$  A $\beta_{1-40}$  at which clusters were slightly prolonged (Table 1).

The results confirm that A $\beta$  at picomolar and low nanomolar concentrations can trigger activation of  $\alpha$ 7 channels.

### Amyloid- $\beta$ Peptides Decrease the Duration of $\alpha$ 7 Activation Episodes

We next explored the effect of A $\beta$  on ACh-elicited channels. To this end, we recorded channels activated by  $100 \mu\text{M}$  ACh in the presence of A $\beta_{1-42}$  ( $10$  and  $100 \text{ nM}$ ). The mean durations of openings and bursts at both A $\beta$  concentrations were slightly briefer than the control but the differences were not statistically significant (Table 2). Due to the very brief durations, differences on these values may be inaccurate since they approach the resolution limit of our system. We therefore tested the effect of A $\beta$  ( $10$ – $1,000 \text{ nM}$ ) on ACh-activated channels in the presence

**TABLE 2 |** Effect of A $\beta$  on ACh-elicited channel activity.

PAM	A $\beta$	A $\beta$ (nM)	n	Mean open duration (ms)	Mean cluster/burst duration (ms)
No	No	0	3	$0.36 \pm 0.07$	$0.77 \pm 0.21$
	A $\beta_{1-42}$	10	3	$0.26 \pm 0.02$	$0.48 \pm 0.06$
		100	3	$0.27 \pm 0.04$	$0.49 \pm 0.11$
PNU-120596	No	0	6	$159.6 \pm 69.6$	$2263 \pm 990$
	A $\beta_{1-40}$	50	3	$176.7 \pm 22.5$	$2220 \pm 256$
		100	5	$61.6 \pm 33.1^*$	$698 \pm 409^*$
		1,000	3	$23.4 \pm 22.0^*$	$931 \pm 303^*$
	A $\beta_{1-42}$	10	5	$61.1 \pm 23.8^*$	$888 \pm 261^*$
		100	5	$54.7 \pm 47.8^*$	$755 \pm 560^*$
1,000		6	$59.6 \pm 32.2^*$	$883 \pm 289^*$	
NS-1738	No	0	12	$4.2 \pm 1.8$	$31.1 \pm 8.8$
	A $\beta_{1-40}$	100	10	$2.7 \pm 1.1^*$	$8.4 \pm 4.8^{****}$
	A $\beta_{1-42}$	100	4	$2.1 \pm 1.0^*$	$10.7 \pm 2.4^{****}$

Single-channel recordings from cells expressing human  $\alpha$ 7 were recorded in the presence of  $100 \mu\text{M}$  ACh,  $100 \mu\text{M}$  ACh plus  $1 \mu\text{M}$  PNU-120596 or  $100 \mu\text{M}$  ACh plus  $10 \mu\text{M}$  N-(5-Chloro-2-hydroxyphenyl)-N'-[2-chloro-5-(trifluoromethyl) phenyl]urea (NS-1738). The effects of A $\beta$  was evaluated for all conditions. The mean open and cluster durations were obtained from the corresponding histograms. Statistical comparisons were performed against the corresponding control condition using ANOVA (Dunnett's multiple comparisons test). \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ .

$1 \mu\text{M}$  PNU-120596, which by increasing the duration of the activation episodes allows a better description of the molecular effects.

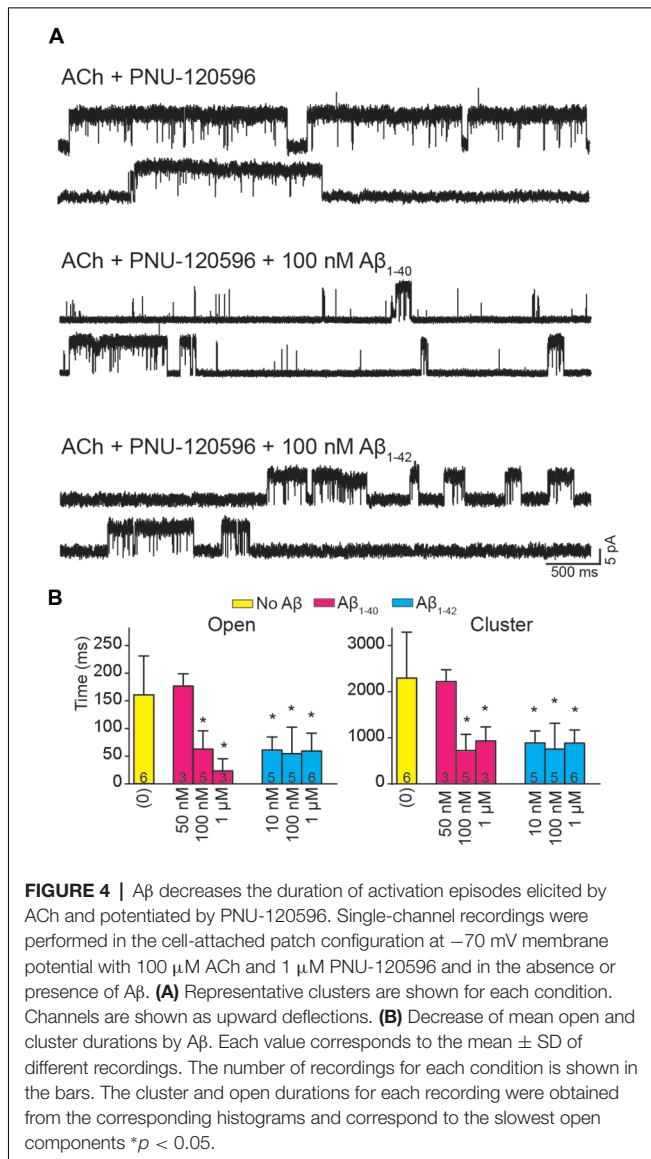
The presence of A $\beta_{1-40}$  or A $\beta_{1-42}$  reduced the mean duration of openings and clusters elicited by ACh and potentiated by PNU-120596 as a function of concentration (Figure 4A). Whereas  $50 \text{ nM}$  A $\beta_{1-40}$  did not affect open and cluster durations,  $10 \text{ nM}$  A $\beta_{1-42}$  reduced these durations. The mean open and cluster durations were statistically significantly briefer than the control at concentrations equal and higher than  $100 \text{ nM}$  for A $\beta_{1-40}$  and  $10 \text{ nM}$  for A $\beta_{1-42}$  ( $p < 0.05$ , Table 2). At these concentrations, the mean durations were reduced about 3-fold (Figure 4B).

To further determine if the effect of A $\beta$  on  $\alpha$ 7 potentiation is specific for type II PAMs, we evaluated the action on channels activated by ACh and potentiated by NS-1738, which is a type I PAM (Timmermann et al., 2007; Andersen et al., 2016). In the presence of ACh, NS-1738 ( $10 \mu\text{M}$ ) increased the mean open duration from about  $0.3 \text{ ms}$  to about  $4 \text{ ms}$  and opening events appeared grouped in bursts of about  $30 \text{ ms}$  (Figure 5, Table 2). In the presence of  $100 \text{ nM}$  A $\beta_{1-40}$  or A $\beta_{1-42}$ , the mean burst duration was reduced to about 3-fold and the mean open duration was also statistically significantly briefer than in the control condition (Figure 5, Table 2).

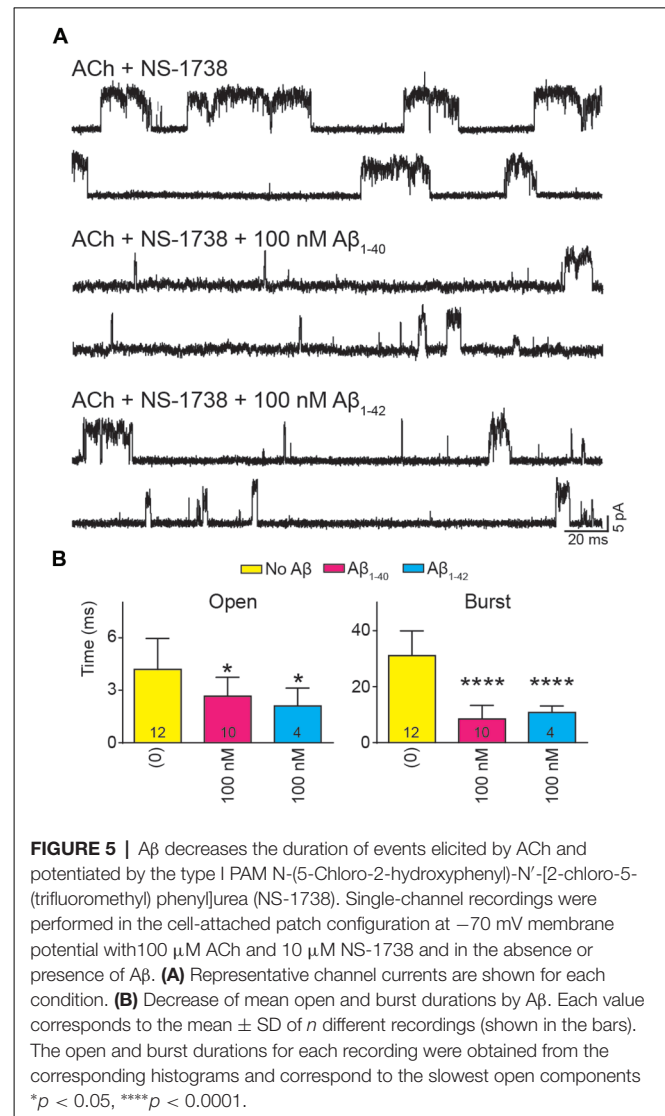
Thus, we conclude that episodes elicited by ACh and potentiated by both types of PAMs are reduced by A $\beta$  at high nanomolar concentrations.

## DISCUSSION

Low concentrations (picomolar) of soluble A $\beta$  peptides in the brain of healthy people have been reported to play physiological roles whereas in AD patients concentrations increase to the nanomolar range and trigger the formation of insoluble plaques,



a major neuropathologic hallmark of AD (Dineley, 2007; Parihar and Brewer, 2010; Collins-Praino et al., 2014; Puzzo et al., 2015). However, the possibility that A $\beta$  oligomers play an important role in AD progression has gained weight (Miñano-Molina et al., 2011; Puzzo et al., 2015). Moreover, in the absence of plaques, intraneuronal accumulation of A $\beta$  peptide has been shown to correlate with the initial steps in the tau-phosphorylation cascade, alterations in ERK2 signaling and impairment of higher CNS functions in rats (Echeverria et al., 2004a,b). Another feature of AD is the severe cholinergic deficit, which involves mainly  $\alpha$ 4 $\beta$ 2 and  $\alpha$ 7 receptors. It has been established that  $\alpha$ 7 exhibits an exceptionally high A $\beta$  affinity, an interaction that may influence synaptic transmission and plasticity and may also contribute to A $\beta$ -mediated synaptic neural network dysfunction and to the severe cholinergic deficit (Wang et al., 2000; Buckingham et al., 2009; Puzzo et al., 2011; Dineley et al., 2015). Several reports have described the intracellular pathways



involved in A $\beta$  toxicity as well as crosstalk between A $\beta$ - and  $\alpha$ 7-triggered signaling pathways (see review in Buckingham et al., 2009; Dougherty et al., 2003; Parri et al., 2011). However, the molecular mechanism by which A $\beta$  affects  $\alpha$ 7 ionotropic activity is not well understood and has been explored mainly at the macroscopic current level. Thus, we took advantage of two different approaches to decipher the molecular basis of the direct actions of oligomeric A $\beta$  at  $\alpha$ 7. Our spectroscopic results revealed that  $\alpha$ 7 adopts distinct stable conformations depending on the A $\beta$  concentration range, and our single-channel recordings revealed that A $\beta$  triggers  $\alpha$ 7 channel openings at low concentrations (picomolar to low nanomolar range) whereas at high concentrations (nanomolar to low micromolar range) it decreases the duration of ACh-elicited activation episodes. Both results fully support the idea that A $\beta$  can act as an agonist and a negative modulator of  $\alpha$ 7 at different, physiologically attainable, concentrations.

One of the main concerns of working with A $\beta$  is related to the standardization of the oligomeric preparations since

aggregation is a dynamic and complex process, which is highly sensitive to preparation, experimental and analyzing conditions (Bitan et al., 2005; Buckingham et al., 2009; Hayden and Teplow, 2013; Watt et al., 2013). Thus, preparations are usually heterogeneous since many types of soluble species co-exist. However, this scenario mimics the physiological/pathological situations where different oligomeric species and fibrils co-exist in a dynamic equilibrium (Walsh et al., 2000; Shankar et al., 2008; Noguchi et al., 2009; Santos et al., 2012; Esparza et al., 2016). Nevertheless, the results obtained with different A $\beta$  oligomer preparations have yielded rather consistent results (Palop and Mucke, 2010). In particular, the effects exerted by our A $\beta$  preparations, which are compatible with a heterogeneous array of oligomers and the absence of fibrils, are in general agreement with previous reported effects of oligomeric preparations on  $\alpha$ 7 (Liu et al., 2001; Dineley et al., 2002; Khan et al., 2010; Tong et al., 2011).

$\alpha$ 7 ionotropic activity is characterized by very brief, sub-millisecond opening events and rapid desensitization (Bouzat et al., 2008; Corradi and Bouzat, 2016). We found that A $\beta$  is capable of eliciting  $\alpha$ 7 activity either in the absence or presence of the type II PAM PNU-120596. In both conditions, activation by A $\beta$  was favored at 100 pM or low nanomolar concentrations and was not detected at micromolar concentrations, in close agreement with macroscopic recordings of  $\alpha$ 7 expressed in oocytes (Dineley et al., 2002, 2015). Channel activity elicited by A $\beta$  was significantly reduced with respect to that elicited by ACh in terms of the number of active patches and frequency of opening events. However, once opened, the mean open duration, cluster architecture and mean cluster duration were similar to those of ACh-elicited channel activity.

Conformational changes of  $\alpha$ 7 driven by the sole presence of A $\beta$  at a low concentration were sensed by CrV. At 100 pM, oligomeric A $\beta$  drove  $\alpha$ 7 conformation towards that of desensitized receptors but the subsequent addition of Carb allowed further conformational changes. Although this method senses conformations under equilibrium and cannot provide information about CrV  $K_D$  for open channels, the fact that in the presence of A $\beta$   $\alpha$ 7 is still responsive to Carb is compatible with the induction of both active and desensitized states. Nevertheless, the conformation of  $\alpha$ 7 in the presence of low A $\beta$  and high Carb concentrations is different to that of the desensitized state (high Carb alone) as sensed by CrV, thus indicating the induction of a novel conformational state.

The enhancement of  $\alpha$ 7 activity as a protective role in AD and for the treatment of cognitive and memory impairment associated to neurological disorders appears to be well established (Inestrosa et al., 2013; Lendvai et al., 2013; Wallace and Bertrand, 2013; Uteshev, 2014; Dineley et al., 2015; Corradi and Bouzat, 2016; Yang et al., 2017). Also, a protective, physiological role has been proposed for soluble A $\beta$  at low concentrations in healthy individuals (Giuffrida et al., 2009; Puzzo et al., 2015). Thus, the capability of low concentration A $\beta$  preparations to activate  $\alpha$ 7 could be related to beneficial physiological effects. However, it would be expected that long-term exposure of an activator would lead to receptor desensitization.

Several reports have shown that high concentrations of A $\beta$  have an inhibitory effect on the amplitude of  $\alpha$ 7-activated macroscopic currents as well as on signaling pathways (Dineley et al., 2002; Parri et al., 2011). In close agreement, we found that in the presence of PAMs the mean duration of activation episodes (clusters or bursts), which arise from a single receptor molecule, as well as the open channel lifetime are significantly reduced by high concentrations of oligomeric A $\beta$ . There was also a trend of reduced mean durations in the absence of PAMs, but values were not statistically significantly different to the control. However, due to the very brief durations, which are close to the time-resolution limit of our system, such reduction may be underestimated. From a mechanistic point of view, the decreased duration of activation episodes and openings may be compatible with increased desensitization and/or channel block. We can discard fast open-channel blockade since brief closings corresponding to blocked openings (flickering) were not detected. However, enhanced desensitization and slow channel block processes are difficult to distinguish by electrophysiological techniques (Arias et al., 2009; Bouzat and Sine, 2018). CrV experiments showed that at high A $\beta$  concentrations  $\alpha$ 7 adopts a conformational state which is not different to the resting state in terms of CrV  $K_D$  values and from this state  $\alpha$ 7 is not further reactive to Carb. In agreement with these observations, it was shown for the muscle nAChR that the channel blocker QX-314 does not change the CrV  $K_D$  value of the resting state and that this value remains constant even in the presence of Carb (Fabiani et al., 2018). Thus, we can infer that oligomeric A $\beta$  at high concentrations behave as a slow channel blocker of  $\alpha$ 7. The reduced  $\alpha$ 7 activity in the presence of A $\beta$  may contribute to the cholinergic signaling deficit and thus may be involved in the initiation and development of AD.

The combined action of PAMs and A $\beta$  suggests that  $\alpha$ 7 potentiation by PAMs would be probably lower than expected in AD patients. By macroscopic current recordings it has been shown that A $\beta$ <sub>1-42</sub> inhibits  $\alpha$ 4 $\beta$ 2 and  $\alpha$ 2 $\beta$ 2 receptors and this inhibition is prevented in the presence of a PAM (Pandya and Yakel, 2011). These results are not in full disagreement with ours because, though reduced, we still detected potentiation. The characterization of the influence of A $\beta$  on  $\alpha$ 7 potentiation contributes to a better extrapolation of the molecular effects of PAMs to their potential therapeutic effects.

A collateral but still important result of our study is the demonstration of different CrV binding profiles between  $\alpha$ 7 and muscle nAChRs. In the muscle nAChR, the CrV affinity for the desensitized state is greater than for the resting state whereas in  $\alpha$ 7 it is the other way around. CrV binds to luminal non-competitive antagonist sites which are localized in the channel vestibule (Lurtz and Pedersen, 1999). Our results showed that the conformation of this region is similar for both receptors in the resting state, but it is different in the desensitized state. Thus, the structural arrangements induced by prolonged exposure to Carb are different between muscle and  $\alpha$ 7 nAChRs. Overall, the use of a conformational probe has proved to be useful for revealing receptor subtype specific structural arrangements associated with functional states and opens doors to further studies in this respect.



The binding site of A $\beta$  remains undefined. Computer docking studies suggested that A $\beta$  may interact with  $\alpha$ 7 at agonist binding-site interfaces (Espinoza-Fonseca, 2004) and the conserved tyrosine 188 at Loop C of the agonist binding site was proposed to be involved in  $\alpha$ 7 activation by A $\beta$  (Tong et al., 2011). On the other hand, the transmembrane cavity, a binding site of different allosteric ligands in the Cys-loop receptor family (Gill et al., 2011; Sauguet et al., 2015; Corradi and Bouzat, 2016), was proposed to be involved in the noncompetitive block of A $\beta$  on  $\alpha$ 4 $\beta$ 2 (Pandya and Yakel, 2011). Our spectroscopic results demonstrating that in the presence of low A $\beta$  concentrations  $\alpha$ 7 is still reactive to Carb suggest that A $\beta$  does not occupy orthosteric agonist binding sites and, in consequence, activation may be mediated by allosteric sites. Allosteric activation of  $\alpha$ 7 through a transmembrane site has been shown for 4BP-TQS. However, the activity profile is strikingly different to that of ACh, which is not the case of A $\beta$  (Gill et al., 2011; Lasala et al., 2018). Alternatively, A $\beta$  may not occupy the five orthosteric agonist sites but may still be able to induce activation; the remaining sites, subsequently occupied by Carb, may favor activation and desensitization. This possibility is in line with our previous reports showing that occupancy of only one of the five ACh-binding sites is required for  $\alpha$ 7 activation (Andersen et al., 2013). At high concentrations, A $\beta$  may probably inhibit  $\alpha$ 7 by acting through an allosteric site, different from that of CrV since it does not interfere with its binding. Dual actions as low-efficacy agonists and channel blockers have been described for several compounds acting at different sites of nAChRs (reviewed in Bouzat and Mukhtasimova, 2018; Bouzat and Sine, 2018).

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Overall, our study provides information from a molecular perspective to understand A $\beta$  complex actions at the higher cellular level.

## AUTHOR CONTRIBUTIONS

ML, CF, JC, SA and CB contributed to study design, analysis and interpretation of data. ML and CF: acquisition of data. CB and SA contributed to writing.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fncel.2019.00037/full#supplementary-material>

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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