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Authors: H. Rheinallt Parri, Caterina M. Hernandez, Kelly T. Dineley



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**RESEARCH UPDATE: ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR MECHANISMS IN
ALZHEIMER'S DISEASE**

H. RHEINALLT PARRI¹, CATERINA M. HERNANDEZ², AND KELLY T. DINELEY²

¹School of Life and Health Sciences, Aston University, Birmingham, UK; ²Department of
Neurology, University of Texas Medical Branch, Galveston, Texas, USA;

CORRESPONDING AUTHOR – Kelly T. Dineley, Ph.D.
Department of Neurology
University of Texas Medical Branch
Galveston, TX, USA
77555-0616
409-747-7060
ktdinele@utmb.edu

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ABSTRACT

Aberrant amyloid- β peptide ($A\beta$) accumulation along with altered expression and function of nicotinic acetylcholine receptors (nAChRs) stand prominently in the etiology of Alzheimer's disease (AD). Since the discovery that $A\beta$ is bound to α 7 nAChRs under many experimental settings, including post-mortem AD brain, much effort has been expended to understand the implications of this interaction in the disease milieu. This research update will review the current literature on the α 7 nAChR – $A\beta$ interaction *in vitro* and *in vivo*, the functional consequences of this interaction from sub-cellular to cognitive levels, and discuss the implications these relationships might have for AD therapies.

1. INTRODUCTION

Alzheimer's disease (AD) is marked by selective cholinergic denervation of the cerebral cortex which is most severe in the temporal lobes and the adjacent limbic and paralimbic areas. The hippocampus is a particularly early and vulnerable target of the disease. These neocortical cholinergic pathways are critical for the modulation of attention and memory; as such, the AD cholinergic lesion manifests as episodic memory impairment (1-4). The clinical observations that cholinomimetics induce symptomatic improvement in AD and the correlation between the magnitude of cholinergic depletion and the severity of dementia provides clinical evidence for the relevance of the cholinergic lesion to the clinical features of AD (5-8).

The basal forebrain, including the medial septal nucleus, diagonal band nuclei, and nucleus basalis, is the major source of cholinergic input to the hippocampus and neocortex. The $\alpha 7$ subtype of nicotinic acetylcholine receptors (nAChRs) is particularly enriched in these cholinergic target areas; in fact, initial A β deposition in early AD overlap with $\alpha 7$ nAChR expression in the basal forebrain cholinergic system (9, 10). Furthermore, the cholinergic deficit in early AD is due in part to altered expression and function of these receptors (8, 11-16). The $\alpha 7$ nAChRs flux the pluripotent second messenger Ca²⁺ and have been shown to modulate neuron excitability, neurotransmitter release, the induction of LTP, learning, and memory (17-21). Likewise, in patients with mild to moderate AD, activation of this receptor improves attention, learning, and memory performance (22-26). Therefore $\alpha 7$ nAChRs are highly implicated in the etiology of early AD.

In the decade-plus since the discovery of a high affinity interaction between A β peptides and $\alpha 7$ nAChRs, several investigative teams have aggressively pursued the biological relevance

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4 of this interaction. At this time, these efforts support a model in which the $\alpha 7$ nAChR – A β
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6 interaction performs a physiologic role since A β peptides are continuously produced under
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8 normal conditions as well as contributes to the etiology of AD as A β peptide concentration and
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10 aggregation proceed pathologically (9, 10, 27-30). This research update will discuss the current
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12 literature on the $\alpha 7$ nAChR – A β interaction *in vitro* and *in vivo*, the functional consequences of
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14 this interaction from sub-cellular to cognitive levels, and discuss the implications these
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16 relationships have for AD therapies.
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22 **2. A β PEPTIDES ARE CONFORMATIONALLY DYNAMIC**

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25 *In vivo* generated A β fragments can be of different lengths and can take many forms, all
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27 of which may behave differently in biological systems. A β in a monomeric form is relatively
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29 unstructured *in vitro*. Oligomerization (dimers, trimers, tetramers, hexamers, dodecamers, etc)
30
31 can make the fragment more rigid while retaining its aqueous solubility. Further aggregation of
32
33 A β can create an insoluble fibril structure, which is a key component of the amyloid plaques
34
35 found in individuals with AD. While it is widely agreed that purely monomeric and fibrillar
36
37 assemblies of A β peptide are unlikely to be the disease-relevant stoichiometries, which
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39 oligomeric aggregate species is responsible for the synaptic dysfunction and ultimate
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41 neurodegeneration in AD remains debated.
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48 Several lines of evidence indicate that oligomeric assemblies of A β possess unique
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50 functional properties including the ability to modulate synaptic transmission and influence
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52 learning and memory in an $\alpha 7$ nAChR-dependent manner. For example, purified oligomers
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54 (dimers, trimers, as well as a 56 kDa dodecamer aggregate) of *in vitro* and *in vivo* produced A β
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56 can disrupt synaptic plasticity and cognitive function when administered at high (nanomolar)
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58 concentration and $\alpha 7$ nAChR activation can overcome LTP impairments suggesting that $\alpha 7$
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4 nAChRs are an important target of oligomeric A β (31-35). However, recent work has implicated
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7 very low (picomolar) concentrations of monomer, trimer, tetramer, and hexamer A β_{1-42} as
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10 playing a role in modulating hippocampal synaptic plasticity and enhancing cognitive function in
11
12 mice via an $\alpha 7$ nAChR-dependent mechanism (36, 37). How can one observe such conflicting
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14 effects of $\alpha 7$ nAChR – A β interaction? Given that A β peptide structure and aggregation
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16 properties are dynamic and depend on concentration, pH, salinity, chelation status, and
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18 temperature, it is not surprising that very different results are obtained with A β solutions
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20 prepared in methodologically distinct ways.
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25 The work reviewed here exclusively used soluble A β peptides that likely represent a
26
27 mixture of monomeric and oligomeric assemblies. However the precise structure and
28
29 aggregation state of the peptide solution in these studies is largely unknown, further
30
31 confounding the interpretation of results. Therefore, it will continue to be important to not
32
33 only specify the concentration of soluble A β used, but also to specify the structural nature of
34
35 the preparation. This is currently becoming much more of a trend and several recent
36
37 publications have attempted to structurally define the peptide solution (36, 38-40). These
38
39 efforts greatly facilitate our interpretation of investigations into the complex nature of A β
40
41 peptide interaction with $\alpha 7$ nAChRs. It is hoped that future work will correlate the effects of
42
43 the A β – nAChR interaction with specific peptide structures; as has been formally requested in a
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45 recent editorial (Cole, Nature Neuroscience, 2011).
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53 **3. $\alpha 7$ nAChRs AND A β INTERACT WITH HIGH AFFINITY *IN VITRO* AND *IN VIVO***

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56 An $\alpha 7$ nAChR – A β interaction was first described over a decade ago; since then many
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58 studies have reported seemingly incongruent consequences of this interaction emphasizing a
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60 complex biology that underlies this interaction. Initial work published by Wang *et al.* (27, 28)
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4 demonstrated that α 7 nAChRs and A β are co-localized in AD cortical regions including the
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6 hippocampus; these proteins are found not only in the membrane fraction but also in amyloid
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8 plaque deposits. They also demonstrated that the receptor – peptide complex could be co-
9
10 immunoprecipitated and detected with immunoblot; this was shown for both control and AD
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12 brain samples. Because this association resists detergent treatment, it prompted the
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14 investigators to postulate that α 7 nAChRs and A β may associate with rather high affinity,
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16 possibly for extended periods of time (27, 41). As discussed in the following sections, this
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18 hypothesis has yet to be refuted and more recent findings indicate that A β -mediated
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20 inactivation of α 7 nAChRs may be one of the detrimental aspects of this protein interaction in
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22 AD (9, 10, 42).
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30 **3.1 NATURE OF A β BINDING TO α 7 nAChRs**

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32 The exact nature of the A β interaction with α 7 nAChRs is not well understood.
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34 Computer simulated docking studies have been performed by Espinoza-Fonseca (2004) utilizing
35
36 the homology model of the human α 7 nAChR derived from the X-ray structure of the
37
38 acetylcholine-binding protein (AChBP) and the lowest energy NMR structure of the human A β ₁₋₄₂
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40 peptide as well as four fragments (amino acids 1-11, 10-20, 12-28, and 22-35) (43, 44).
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43 These analyses were achieved using a modified version of ESCHER software that analyzes the
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45 complementarity of the target and probe proteins in 360° using their solvent accessible
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47 surfaces. Their results indicated that the full length peptide and peptide fragments bind
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49 parallel to the receptor within the binding interface between two subunits. Based on the series
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51 of docking studies with the full length A β peptide and the four fragments in complex with the
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53 α 7 nAChR, Espinoza-Fonseca concluded that the interaction domains common amongst all five
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55 receptor:peptide complexes involved residues V12-K28 of A β ₁₋₄₂ and the agonist binding loop C
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4 of one subunit of the receptor and a loop delineated by amino acids 62-74 and loop G of the
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6 adjacent subunit of the receptor. Thus, it appears energetically favorable for monomeric A β ₁₋
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9 42 to bind components of the agonist binding site.

10
11 To further support the strength of these studies, the fragments used in the Espanoza-
12
13 Fonseca studies correspond to the fragments identified by Wang et al. (2003) that were most
14
15 effective in competition binding studies against cells expressing $\alpha 7$ nAChRs (45). The A β ₁₋₄₂
16
17 peptide fragments defined as interaction points with the ligand binding domain of the $\alpha 7$
18
19 nAChR are common between both A β ₁₋₄₀ and A β ₁₋₄₂ suggesting that the two additional
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21 hydrophobic amino acids at the C-terminus of A β ₁₋₄₂ alter the conformation of the central
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23 hydrophilic portion of the peptide to increase binding affinity (28).
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30 Initial competition binding studies on membrane preparations from brain regions and
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32 cell lines expressing the $\alpha 7$ nAChR indicated that A β association occurred with an affinity in the
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34 low picomolar range while similar experiments for $\alpha 4\beta 2$ nAChRs indicated an affinity 100 –
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36 5,000 times lower (27, 28). The apparent affinity for the $\alpha 7$ nAChR – A β ₁₋₄₂ interaction and the
37
38 fact that soluble A β ₁₋₄₂ in healthy brain and CSF has been estimated at picomolar values
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40 indicates that these two proteins could associate under normal physiologic conditions leading
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42 to receptor activation (46-48); recent behavioral and synaptic plasticity studies provide
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44 evidence that this is indeed the case, at least in hippocampus (36, 37). As AD progresses A β
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46 levels exponentially increase in AD-affected brain regions, achieving nanomolar levels and, as
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48 discussed in later sections, likely lead to $\alpha 7$ nAChR inactivation (46-48).
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56 Curve fit analysis from binding studies on $\alpha 7$ -expressing cells, suggested that there were
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58 two saturable A β binding sites (28). While extensive binding studies with A β peptides and $\alpha 7$ -
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60 expressing cells have not since been published, several functional studies utilizing a variety of
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4 preparations have pharmacologically blocked A β -mediated effects via α 7 nAChR-selective
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6 antagonists such as α -bungarotoxin (BTX) and methyllycaconitine (MLA) (31, 36, 39, 49-54).
7
8 These publications utilized A β ₁₋₄₂ preparations that were either roughly defined as ‘oligomeric’
9
10 or were more precisely defined as being comprised of a mixture of at least two of the following:
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12 monomer, dimer, trimer, and hexamer. These data suggest that A β preparations that result in
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14 α 7 nAChR activation utilize a binding site that is comprised, at least in part, by components of
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16 the Cys-loop inter-subunit agonist binding site.
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22 It should be noted that a number of those that have observed A β -mediated functional
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24 antagonism of α 7 nAChRs have reported that this is noncompetitive in nature since α 7 nAChR-
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26 selective antagonists such as BTX or MLA were unable to block, or agonist was able to only
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28 partially overcome A β inhibitory effects (55-58). These noncompetitive binding results infer
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30 that the A β preparations utilized (uncharacterized in these studies) gained access to a binding
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32 site distinct from the Cys-loop inter-subunit acetylcholine ligand binding pocket.
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38 A recently described intra-subunit allosteric binding pocket located within the
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40 transmembrane domain of the α 7 nAChR provides a potential structure-function mechanism to
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42 explain noncompetitive A β antagonism (59-61). In support of this model, recent work on
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44 heterologously expressed α 4 β 2 and α 2 β 2 nAChRs showed that the positive allosteric modulator
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46 desformylflustrabromine relieves the noncompetitive A β ₁₋₄₂ blockade (62). It will be an
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48 important (and challenging) effort to delineate the structural and conformational parameters
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50 that yield competitive and noncompetitive A β – α 7 nAChR interaction.
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56 **4. The nAChR - A β INTERACTION LEADS TO RECEPTOR ACTIVATION AND RECEPTOR INHIBITION**

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58 The evidence for an inhibitory versus a stimulatory role for A β on α 7 nAChRs is fairly
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60 equally divided in the literature (**Table I**). Superficially, these reports appear contradictory;
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4 however potential underlying issues regarding the origin of the receptor populations and
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6 specifics of the biological preparations as well as detection methods differ amongst laboratories
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8 will be discussed. Differing effects of A β on $\alpha 7$ nAChRs indicate that the details regarding cell
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10 type, subcellular location, subunit stoichiometry, accessory protein population, lipid
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12 composition, and post-translational modifications of the receptor may significantly influence
13
14 receptor properties; as has been shown many times previously for this receptor class (63-67).
15
16 In these studies, an equally important, but often overlooked variable is the A β peptide itself. As
17
18 discussed above, early investigations did not characterize the conformation and aggregation
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20 state of the A β peptide stock solutions utilized. However this is changing; some studies
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22 discussed below included this information and will be discussed in terms of the results
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24 obtained.
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32 **4.1 $\alpha 7$ nAChR ANTAGONISM**

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35 The first indication that A β_{1-42} and nAChRs *functionally* interact demonstrated that A β_{1-42}
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37 inhibited nAChR currents recorded from GABAergic interneurons in acutely prepared rat
38
39 hippocampal slices (68). Caged-carbachol-induced currents measured with whole cell
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41 recordings were maximally inhibited (39%) with a dose of 500 nM peptide; doses as low as 100
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43 nM were effective. These effects were rapidly reversible under whole-cell recording conditions.
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46 One of the A β -sensitive channels they characterized using patch clamp recordings was sensitive
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48 to the $\alpha 7$ -selective antagonist MLA. Thus, rat hippocampal interneurons possess $\alpha 7$ nAChRs
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50 that are reversibly antagonized by A β_{1-42} via a mechanism that decreases the probability of
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52 opening.
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59 Subsequent work from this same group studied A β_{1-42} effects on nAChRs expressed in
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61 *Xenopus* oocytes (57). Co-application of a maximal dose of carbachol and 1 μ M A β_{1-42} to
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4 oocytes expressing rat $\alpha 7$ nAChRs resulted in no effect on $\alpha 7$ nAChRs currents in contrast with
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6 their previous findings in hippocampal interneurons; further suggesting that the *in situ*
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8 environment, such as the cell system utilized for expression, can significantly alter receptor
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10 properties (69, 70).
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14 $\alpha 7$ nAChR antagonism was also observed in rat hippocampal neuronal cultures, it was
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16 demonstrated that the response of both somato-dendritic and presynaptic $\alpha 7$ nAChRs was
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18 rapidly and almost completely blocked by exposure to 100 nM $A\beta_{1-42}$ and they reported an IC_{50}
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20 value of 7.5 nM (56). Full recovery occurred within 5 min of washout. This functional
21
22 antagonism appeared noncompetitive from [^{125}I]-BTX binding assays. Additional experiments
23
24 demonstrated that $A\beta$ blockade was voltage-independent, did not result from open channel
25
26 block, and likely resulted from interaction with the N-terminal extracellular domain of the
27
28 receptor. Furthermore, it was determined that intracellular Ca^{2+} and G-protein activity was not
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30 necessary for inhibition of $\alpha 7$ nAChR function by $A\beta_{1-42}$, suggesting that prior receptor
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32 activation did not mediate antagonism.
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40 Pym *et al.* (58) expressed human $\alpha 7$ nAChRs in *Xenopus* oocytes and found these
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42 receptors to be antagonized by $A\beta_{1-42}$ (and $A\beta_{1-40}$). Maximum acetylcholine currents were
43
44 inhibited approximately 50% in the presence of 10 nM of either peptide. Given that binding
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46 studies indicated that $A\beta_{1-42}$ exhibits higher affinity than $A\beta_{1-40}$ suggests that, at this
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48 concentration, the dose-response curves overlap. Concentrations in the range of 1 pM – 100
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50 nM were tested and failed to activate the receptor. Similar to the above described studies, Pym
51
52 *et al.* (58) found that $A\beta$ effects were reversible. Grassi *et al.* (55) also reported that human $\alpha 7$
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54 nAChRs expressed in *Xenopus laevis* oocytes were antagonized by 100 nM $A\beta_{1-42}$. Antagonism
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56 was non-competitive and an analysis of a dose response study for inhibition of currents elicited
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4 by 100 μM ACh yielded an IC_{50} value of 90 nM. Attempts to activate these receptors with 10
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7 nM $\text{A}\beta_{1-42}$ were unsuccessful. Taken together, this set of studies suggests that soluble (human)
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9
10 $\text{A}\beta$ does not activate human $\alpha 7$ nAChRs and are noncompetitively antagonized by $\text{A}\beta$ peptides.
11
12 Again it must be emphasized that the conformation and aggregation state of the $\text{A}\beta$
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14
15 preparations used in the studies above is unknown.

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17 A contrasting result was recently obtained when human neuroblastoma cells that
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19
20 express $\alpha 7$ nAChRs were exposed to human oligomeric $\text{A}\beta_{1-42}$ (39). The results from Young et
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22
23 al. (2009) were very similar to a series of studies from Dineley's group utilizing rat hippocampal
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26 slices and both human and rat oligomeric $\text{A}\beta_{1-42}$ (38, 50). Concentrations in the picomolar to
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28
29 nanomolar range of oligomeric $\text{A}\beta_{1-42}$ applied to SY5Y neuroblastoma cells resulted in ERK
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32 MAPK activation and this was blocked by the competitive antagonists MLA and BTX (39). These
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35 findings again emphasize that the conformation and aggregation state of the $\text{A}\beta$ peptide
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38 preparation is a critical factor in the study design.

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40 The results discussed above were obtained from homomeric $\alpha 7$ nAChRs based upon
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43 receptor kinetics and pharmacological properties (*in situ* recordings) or through heterologous
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46 expression in *Xenopus* oocytes, for example. An $\alpha 7$ -containing heteromeric nAChR was
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48
49 described a short time ago by Drs. Wu and Lukas in basal forebrain cholinergic neurons (71). In
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51
52 this study, it was demonstrated that nAChRs expressed on freshly dissociated cholinergic
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55 medial septum/ diagonal band (MS/DB) neurons exhibit mixed kinetic and pharmacological
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58 properties of $\alpha 7$ - and $\beta 2$ -containing nAChRs. These 'mixed' properties were absent in MS/DB
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60
61 neurons prepared from $\beta 2$ nAChR knock-out mice. Functional antagonism of $\alpha 7\beta 2$ nAChRs
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63
64 expressed on cholinergic MS/DB neurons was demonstrated at $\text{A}\beta_{1-42}$ concentrations as low as
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67 1 nM; 100 pM was ineffective. Additionally, it was demonstrated that oligomeric $\text{A}\beta_{1-42}$ more

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4 effectively antagonized these receptors than fibrillar $A\beta_{1-42}$; monomeric $A\beta_{1-42}$ had no effect.
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6 Such selective sensitivity to relatively low concentrations of oligomeric $A\beta_{1-42}$ and the necessity
7
8 of nAChR function in maintenance of the cholinergic phenotype suggests that the selective
9
10 vulnerability of the basal forebrain cholinergic system during early AD may in part be due to
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12 blockade of this heteromeric α 7 β 2 nAChR by oligomeric $A\beta$ assemblies occurring due to AD
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14 progression.
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20 In all cases discussed above in which functional antagonism was reported, inhibition
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22 required at least 1-10 nM $A\beta$ and block was typically incomplete, but at least 30%. These
23
24 studies are best summarized as rat and human α 7 nAChR inhibition by $A\beta_{1-42}$ peptide required
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26 pre-application of the peptide; receptor inhibition was reversible and exhibited noncompetitive
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28 binding properties (55-58, 68, 72).
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32 **4.2 α 7 nAChR ACTIVATION BY OLIGOMERIC ASSEMBLIES OF $A\beta_{1-42}$**

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34 While an interaction between α 7 nAChRs and $A\beta$ peptide is well-established, in the
35
36 presence of $A\beta$, α 7 nAChRs rapidly desensitize making direct electrophysiological recordings a
37
38 challenge. Rat α 7 nAChRs expressed in *Xenopus* oocytes were activated following application of
39
40 femtomolar to nanomolar concentrations of $A\beta_{1-42}$ (54). Analysis of the $A\beta_{1-42}$ preparation by
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42 non-denaturing Tris-Tricine gel electrophoresis indicates that the $A\beta$ used in these experiments
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44 was primarily hexameric oligomers with additional components of trimers and monomers (38).
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46 Receptor activation led to Ca^{2+} influx as evidenced by a reduction in current amplitude when
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48 Ca^{2+} in the recording solution was replaced by Ba^{2+} , thus preventing the activation of the
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50 endogenous Ca^{2+} -activated chloride current that enhances membrane depolarization and
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52 current amplitude. $A\beta_{1-42}$ activation of α 7 nAChRs was blocked by the α 7-selective antagonist
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54 MLA and cross-desensitized by the α 7-selective agonist DMXB [3-(2,4-dimethoxybenzylidene)-
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4 anabaseine], suggesting that the $\alpha 7$ nAChR ligand binding domain and the $A\beta_{1-42}$ binding site at
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6 least partially overlap. The lowest doses that were effective in this study (100 fM – 10 pM) and
7
8 the observation that $A\beta_{1-42}$ was more potent in activating $\alpha 7$ nAChRs than $A\beta_{1-40}$, are consistent
9
10 with the binding studies performed by Wang *et al.* (28) in which it was observed that $\alpha 7$
11
12 nAChRs exhibit higher affinity for $A\beta_{1-42}$ versus $A\beta_{1-40}$. While it was demonstrated that $A\beta$
13
14 peptides could directly activate $\alpha 7$ nAChRs; these currents were small (~200 nA). Furthermore,
15
16 high doses or prolonged exposure to $A\beta_{1-42}$ led to receptor inhibition, possibly through a
17
18 desensitization mechanism (54). This was suggested by the inverted U shape of the dose –
19
20 response curve and the observation that more than one exposure or pre-exposure to $A\beta_{1-42}$ led
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22 to $\alpha 7$ nAChR inactivation. In summary, rat $\alpha 7$ nAChRs expressed by *Xenopus* oocytes are
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24 responsive to physiologically relevant doses of oligomeric $A\beta_{1-42}$; however, these currents are
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26 relatively small indicating that oligomeric $A\beta_{1-42}$ appears to be highly desensitizing leading to
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28 receptor inactivation.
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38 One strategy to overcome the desensitizing nature of $A\beta_{1-42}$ for $\alpha 7$ nAChRs and gain
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40 signal-to-noise in measurements of receptor activation is to exploit the high Ca^{2+} permeability of
41
42 these receptors. As such, $\alpha 7$ nAChR activation commonly leads to Ca^{2+} -induced Ca^{2+} release
43
44 (CICR); voltage-gated Ca^{2+} channels are another component of the overall intracellular Ca^{2+}
45
46 signal in cases where nAChRs evoke significant changes in membrane potential. The Nichols'
47
48 laboratory took advantage of this circumstance by utilizing confocal imaging in combination
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50 with fluorescent Ca^{2+} -indicator dyes to record increases in intracellular Ca^{2+} in isolated
51
52 presynaptic nerve endings purified from rat hippocampus and neocortex (52). These efforts
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54 determined that picomolar $A\beta_{1-42}$ directly led to sustained increases in presynaptic Ca^{2+} via
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56 nAChRs. The effect of $A\beta_{1-42}$ was sensitive to BTX, mecamylamine (MEC), and dihydro- β -
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4 erythroidine (DH β E), indicating the involvement of both α 7-containing and non- α 7-containing
5
6 nAChRs. Interestingly, it was discovered that α 7-containing nAChRs are largely involved in the
7
8 presynaptic actions of A β at picomolar concentrations whereas higher nanomolar
9
10 concentration of A β involves mainly non- α 7-containing nAChRs. Prior exposure of these
11
12 preparations to A β occluded subsequent nicotine-evoked increases in presynaptic Ca²⁺. This
13
14 and the fact that nicotine, albeit at relatively high concentration, could overcome the occlusion
15
16 effect of A β ₁₋₄₂ suggested that the A β and the α 7 nAChR ligand binding site significantly overlap.
17
18 Subsequent studies utilizing presynaptic terminals isolated from the hippocampus and cortex of
19
20 nicotinic receptor knockout mice for either the α 7 or β 2 nAChR subunit, determined that A β -
21
22 mediated increases in intracellular Ca²⁺ were mainly mediated by β 2-containing nAChRs in the
23
24 hippocampus and α 7 nAChRs in the cortex (53, 73). Perhaps the species difference between
25
26 the two studies underlies the failure to detect α 7 nAChR presynaptic involvement in the
27
28 hippocampus of mice. However, yet another study from this group utilized a neuroblastoma
29
30 cell line (NG 108-15) transfected with mouse α 7 nAChR cDNA found that picomolar –
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32 nanomolar soluble A β ₁₋₄₂ induced increased intracellular Ca²⁺ within axonal varicosities that was
33
34 blocked by the α 7 nAChR antagonist, BTX (73). Interestingly, cholesterol depletion with methyl-
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36 β -cyclodextrin significantly attenuated these responses, suggesting that A β -sensitive α 7 nAChRs
37
38 reside within lipid rafts at presynaptic sites. These findings further reinforce that the lipid
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40 composition surrounding nAChR receptor transmembrane domains is an important variable
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42 contributing to nAChR functional profiles (74, 75).
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56 All in all, these studies demonstrate that low concentrations (femtomolar-picomolar) of
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58 oligomeric (e.g., trimer, hexamer) A β ₁₋₄₂ can activate α 7 nAChRs *in situ* and heterologously
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60 expressed in *Xenopus* oocytes. Receptor activation increases intracellular Ca²⁺ and can
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4 potentiate neurotransmitter release. However, higher $A\beta_{1-42}$ concentrations lead to receptor
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6 inactivation, likely through a desensitization mechanism. These results put forth the possibility
7
8 that, under normal physiologic conditions, $A\beta$ and $\alpha 7$ nAChR interaction could lead to receptor
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10 activation *in vivo*, as has been recently demonstrated by Puzzo et al. to be discussed in the next
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12 section (36, 37).
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16 17 **5. FUNCTIONAL CONSEQUENCES OF THE $\alpha 7$ nAChR – $A\beta$ INTERACTION**

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19 Activation of nAChRs causes membrane depolarization and, directly or indirectly,
20
21 increases the intracellular Ca^{2+} concentration. Thus, when nAChRs are expressed on
22
23 presynaptic membranes their activation generally increases the probability of neurotransmitter
24
25 release. When expressed on postsynaptic membranes, nAChR-initiated increases in
26
27 intracellular Ca^{2+} and depolarization activate intracellular signaling mechanisms that contribute
28
29 to neuron homeostasis, synaptic plasticity, learning and memory (for review, see (76). As is the
30
31 case for receptor activation by ACh or nicotine, $A\beta$ activation of $\alpha 7$ nAChRs runs the gamut of
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33 these responses.
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40 **5.1 SIGNAL TRANSDUCTION CONSEQUENCES OF THE $\alpha 7$ nAChR – $A\beta$ INTERACTION**

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42 Since the discovery of an $\alpha 7$ nAChR – $A\beta$ interaction several groups have mapped out
43
44 some of the downstream consequences of this association: Ca^{2+} influx, ERK MAPK activation via
45
46 the PI3K pathway that results in CREB phosphorylation in both a PKA- and Rsk2-dependent
47
48 manner (38, 39, 50, 52, 54, 77). The studies by Dineley *et al.* (50) and Bell *et al.* (38) were
49
50 performed on organotypic hippocampal slice cultures; specificity of the effects occurring via $\alpha 7$
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52 nAChRs was demonstrated with the $\alpha 7$ -selective antagonists MLA and BTX. ERK activation
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54 occurred rapidly and at concentrations as low as 10 pM.
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4 Extended exposure to high (nanomolar) concentration of $A\beta_{1-42}$ led to down-regulation
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6 of ERK MAPK activity; this is also observed in hippocampal samples from aged Tg2576 in which
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8 $A\beta$ is produced in excess from young adulthood onward (38, 50). Interestingly, extended
9
10 exposure to nanomolar $A\beta_{1-42}$ up-regulates $\alpha 7$ nAChRs in hippocampal cultures, comparable to
11
12 the effects of chronic exposure to nicotine (50). Likewise, in the Tg2576 hippocampus $\alpha 7$
13
14 nAChRs continue to up-regulate with age as $A\beta$ accumulates, providing further evidence that $A\beta$
15
16 and $\alpha 7$ nAChRs interact *in vitro* and *in vivo* (50, 78). Dysregulation of $\alpha 7$ nAChRs, ERK MAPK,
17
18 and the downstream transcription factor CREB in the hippocampus of Tg2576 mice occurs
19
20 concomitant with the onset of hippocampus-dependent learning and memory impairments
21
22 (50). These combined *in vitro* findings and *in vivo* observations suggest that, in hippocampus,
23
24 physiological concentrations of $A\beta_{1-42}$ impinge upon signal transduction cascades important for
25
26 cell homeostasis, synaptic plasticity, learning and memory. Short exposure times (minutes) and
27
28 moderate concentrations (picomolar – low nanomolar) do not lead to permanent changes in $\alpha 7$
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30 or the ERK MAPK cascade; higher doses and extended exposure time lead to dysregulation of
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32 $\alpha 7$, ERK MAPK, and CREB accompanied by learning and memory impairments.
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43 Young et al. obtained very similar results to those from Dineley's group utilizing human
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45 SY5Y neuroblastoma cells exposed to human oligomeric $A\beta_{1-42}$. Oligomeric $A\beta$ application
46
47 resulted in ERK MAPK activation and this was blocked by the $\alpha 7$ nAChR competitive antagonist,
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49 MLA, and U0126 compound that inhibits the ERK MAPK upstream kinase, MEK (39).
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53 Utilizing primary neuronal cultures prepared from mouse cortex and hippocampus
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55 Abbot et al. demonstrated that acute exposure to nanomolar (400) $A\beta_{1-42}$ leads to Akt
56
57 phosphorylation via $\alpha 7$ nAChRs (77). Akt is closely associated with PI3K activation which itself is
58
59 involved in signal transduction pathways necessary for neuroprotection as well as synaptic
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4 plasticity, learning and memory (79). nAChRs have long been implicated as playing a role in
5
6 each of these processes; in neuron models for α 7 nAChR-mediated neuroprotection, activation
7
8 of the PI3K-Akt pathway is a crucial downstream effector of nicotine-induced anti-apoptotic
9
10 signaling (80, 81) (82). We previously discussed the work of Dineley et al. and Bell et al., in
11
12 which acute exposure of organotypic hippocampal slice cultures to picomolar-nanomolar $A\beta_{1-42}$
13
14 (and nicotine) led to ERK MAPK activation via α 7 nAChRs (50). Bell et al. (38) further showed
15
16 that $A\beta_{1-42}$ (and nicotine) couples to ERK via PI3K in an α 7 nAChR-dependent manner. Abbott et
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18 al., however, did not test whether PI3K activity was necessary for the Akt phosphorylation
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20 observed following acute $A\beta_{1-42}$ exposure; thus there may exist subtle molecular differences
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22 between the two systems (77).
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30 To summarize, α 7 nAChRs acutely exposed to $A\beta_{1-42}$ leads to activation of signal
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32 transduction cascades associated with neuroprotection, synaptic plasticity, learning and
33
34 memory in an α 7 nAChR-dependent manner (**Figure 1**). The concentration range that was
35
36 effective (picomolar-nanomolar) suggests that endogenous $A\beta$ may serve a modulatory role in
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38 synaptic transmission, plasticity, and even neuroprotection (36, 46, 47, 83-85). Further
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40 discussion on this topic will be covered in the next section.
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45 The fact that α 7 nAChRs also reside on glial cells, notably astrocytes, immediately
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47 implies a major physiological role in astrocytic function, since α 7 nAChRs flux Ca^{2+} , and changes
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49 in intracellular Ca^{2+} are the basis of astrocytic “excitability” (86, 87); (88). These observations
50
51 clearly imply that the relationship between nAChRs and $A\beta$ is a dynamic one and relies on
52
53 several factors such as the *in situ* environment in which the nAChR is expressed (somatic,
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55 dendritic, presynaptic; neuronal, astrocytic, microglial) as well as the *in situ* status of $A\beta$
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57 (concentration, aggregation state, regional distribution).
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5.2 NEUROTRANSMISSION, SYNAPTIC PLASTICITY, LEARNING AND MEMORY

The Nichols' laboratory has performed several studies investigating the ability of soluble A β ₁₋₄₂ to influence neurotransmitter release through activation of presynaptic nAChRs (51, 52, 73). Initial studies demonstrated that soluble A β ₁₋₄₂ activates nAChRs on presynaptic terminals isolated from rat neocortex and hippocampus (52). The observations that A β -induced nAChR activation led to elevation of presynaptic Ca²⁺ and that prior activation of presynaptic nAChRs attenuated subsequent responses to A β ₁₋₄₂ suggested that this interaction could lead to altered synaptic transmission; however this study by Dougherty et al. did not directly test that outcome.

To address whether presynaptic α 7 nAChR – A β interaction impinges upon neurotransmission, the same group then utilized the well-established paradigm for nAChR-mediated dopamine (DA) release from prefrontal cortex to test the hypothesis that A β -activation of presynaptic nAChRs could lead to neurotransmitter release *in vivo* (51); (89). It has been shown previously that both α 7-containing and α 4 β 2 nAChR subtypes are involved in prefrontal cortex DA release; in this study, soluble A β was perfused into mouse prefrontal cortex and the effect on the release of DA outflow via microdialysis was assessed (90, 91). In the presence of tetrodotoxin, A β ₁₋₄₂ at 100 nM evoked the release of DA to ~170% of baseline. A β ₁₋₄₂-evoked DA release was sensitive to antagonists of α 7 nAChRs and was absent in mice in which the α 7 nAChR subunit had been genetically deleted, but was intact in mice harboring a null mutation for the β 2 nAChR subunit (92, 93). Very low relative concentrations (picomolar) of A β ₁₋₄₂ caused a slowly developing and long-lasting depression of DA outflow in the prefrontal cortex. Given that the A β ₁₋₄₂ in these studies was delivered through reverse dialysis, the time to achieve maximal dose is unknown, but likely on the minutes' time scale;

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4 therefore, picomolar $A\beta_{1-42}$ may evoke a low sustained level of presynaptic Ca^{2+} rise, leading to
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6 synaptic depression. It will be of interest if fast delivery of such a concentration of $A\beta_{1-42}$ has
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8 the same effect. Nonetheless, the cumulative work of Dougherty et al. (52) and Wu et al. (51)
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10 provide compelling evidence that $A\beta_{1-42}$, most likely acting through α 7 nAChRs, alters
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12 neurotransmitter release and transmission at certain cortical synapses.
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17 In addition to directly modulating neurotransmitter release, $A\beta_{1-42}$ has additional
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19 synaptic effects by modulating NMDA receptor function through a trafficking mechanism. In
20
21 this study, cortical neuron cultures were used as a cellular model to study glutamatergic
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23 synapses and it was found that exposure to high (μ M) concentrations of $A\beta_{1-42}$ for prolonged
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25 periods of time (\geq 30 minutes), led to NMDA receptor endocytosis in an α 7 nAChR- and Ca^{2+} -
26
27 dependent manner (31). The mechanism involved Ca^{2+} -dependent activation of protein
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29 phosphatase 2B that led to striatal-enriched phosphatase (STEP) dephosphorylation and
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31 activation, which in turn resulted in dephosphorylation of the NR2B NMDA receptor subunit on
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33 a tyrosine residue (Tyr1472, a STEP target). NR2B Tyr1472 dephosphorylation correlated with
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35 receptor endocytosis and depression of NMDA-evoked currents in cortical neuron cultures.
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43 Modeling synaptic deficits of early AD by infusing 300 pmoles of $A\beta_{1-40}$ per day for 11-14
44
45 days into rat hippocampus followed by *in vivo* HFS-induced LTP at Schaffer collateral – CA1
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47 synapses Chen et al. found that α 7 nAChRs were involved in $A\beta_{1-40}$ -induced depression of
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49 synaptic transmission and deficits in LTP (35, 94). Utilizing the α 7 nAChR partial agonist, DMXB,
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51 it was demonstrated that DMXB induced EPSPs were impaired in $A\beta_{1-40}$ infused rat
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53 hippocampus (95). In addition, $A\beta_{1-40}$ infused rats also demonstrated impaired LTP that was
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55 rescued with DMXB. Control experiments demonstrated that: 1) DMXB enhanced *in vivo*
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57 recorded EPSPs in untreated rats; 2) DMXB enhanced EPSPs were blocked by BTX but not $DH\beta E$,
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4 an $\alpha 4\beta 2$ nAChR antagonist; 3) blocking $\alpha 7$ nAChRs with BTX and MLA (but not $\alpha 4\beta 2$ nAChRs
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6 with DH β E) blocked *in vivo* LTP; 4) BTX blocked DMXB enhancement of LTP. Finally, evaluation
7
8 of input/output curves as well as post-tetanic potentiation and paired-pulse facilitation
9
10 suggested that A β_{1-40} infusion leads to diminished presynaptic Ca²⁺ influx that led the authors to
11
12 propose a model in which reduced EPSP in A β_{1-40} infused rats arises from a decline in
13
14 presynaptic glutamate release due to $\alpha 7$ nAChR dysfunction. Collectively, the studies by Chen
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16 et al. suggest that A β -induced blockade of $\alpha 7$ nAChRs can negatively affect synaptic plasticity
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18 and, by extrapolation, possibly learning and memory processes (35, 94).
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25 Gu and Yakel published additional exciting *in vivo* evidence that A β at high (nanomolar)
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27 concentration interacts with presynaptic septal cholinergic $\alpha 7$ nAChRs to affect Schaeffer
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29 collateral (SC) to CA1 plasticity (40). Septal cholinergic stimulation was achieved either by
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31 electrical stimulation or via an optogenetic approach. The type of plasticity depended upon the
32
33 timing of septal cholinergic stimulation relative to the SC input; cholinergic input activated 100
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35 or 10 msec prior to SC stimulation resulted in $\alpha 7$ nAChR-dependent long-term potentiation
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37 (LTP) or short-term depression, respectively. Plasticity was blocked by the $\alpha 7$ nAChR antagonist
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39 MLA and absent in $\alpha 7$ nAChR knockout mice; the $\alpha 4$ nAChR-selective antagonist DH β E had no
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41 effect. Moreover, these two forms of $\alpha 7$ nAChR-dependent plasticity were disrupted by either
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43 10 or 100 nM (but not 1 nM) A β exposure suggesting again that inactivation of $\alpha 7$ nAChRs has
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45 negative effects on synaptic plasticity.
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53 In a series of studies utilizing low (picomolar) concentration of oligomeric A β , Puzzo et
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55 al. have developed the idea that endogenous A β serves as a positive modulator of hippocampal
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57 synaptic transmission via interaction with (presumably) presynaptic $\alpha 7$ nAChRs (36, 37). At the
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59 outset, they demonstrated that 200 pM A β_{1-42} enhanced Schaffer collateral – CA1 LTP using
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4 theta-burst stimulation; enhanced LTP was not achieved if tetanus was not applied nor when a
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6 scrambled peptide was perfused with tetanus, and was absent in hippocampal slices prepared
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8 from $\alpha 7$ nAChR null-mutant mice. Investigations into the mechanism of the $A\beta$ -induced
9
10 enhancement of LTP ruled out that $A\beta$ does not affect spontaneous neurotransmitter release;
11
12 nor did it affect NMDA or AMPA receptor currents. The authors concluded that 200 pM $A\beta_{1-42}$
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14 increases neurotransmitter release in an $\alpha 7$ nAChR-dependent manner during the tetanus.
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16 These results are consistent with a presynaptic effect of $A\beta$ on $\alpha 7$ nAChRs. However, since
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18 astrocytic Ca^{2+} elevations lead to gliotransmitter release, which can then contribute to synaptic
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20 strengthening in the dentate gyrus (96), it is possible glial $\alpha 7$ nAChRs contribute to synaptic
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22 modulation and plasticity. This potential scenario will be discussed in the next section.
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31 In the same set of studies, Puzzo et al. (36) report that 200 pM $A\beta_{1-42}$ delivered through
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33 bilateral cannulae to the dorsal hippocampus also enhances baseline learning and memory in
34
35 wildtype mice. Injections were performed prior to training in the Morris water maze and for
36
37 contextual fear conditioning. Both paradigms are hippocampus-dependent learning and
38
39 memory paradigms; Morris water maze tests spatial navigation learning and memory while
40
41 contextual fear conditioning tests associative learning and memory. In both tasks, mice
42
43 receiving 200 pM $A\beta_{1-42}$ showed improved performance during the testing phase; neither
44
45 wildtype receiving scrambled peptide nor $\alpha 7$ nAChR null mice receiving $A\beta_{1-42}$ showed signs of
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47 cognitive enhancement. Collectively, these findings suggest that $A\beta_{1-42}$ may be an endogenous
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49 neuromodulatory peptide that, at least in hippocampus, utilizes $\alpha 7$ nAChRs to exert its effects.
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57 A second publication from Puzzo et al. tackled this ambitious hypothesis (37). First, it
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59 was demonstrated that hippocampal (but not cerebellum) $A\beta_{x-42}$ level increased following
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61 theta-burst stimulation of Schaffer collaterals and training for contextual fear memory. The
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4 next experiment reduced endogenous rodent A β through antibody depletion and siRNA
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6 methodologies prior to hippocampal LTP induction or training for contextual fear memory. The
7
8 resultant inhibition of LTP and contextual learning lends further support to the model
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10 developed from the set of studies described above. Specifically, antirodent A β antibodies and
11
12 siRNA against rodent APP inhibited Schaffer collateral – CA1 LTP as well as contextual fear
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14 memory; exogenous application of human A β ₁₋₄₂ oligomers (but not monomers) restored
15
16 proper function. Furthermore, A β depletion strategies that diminished both post-tetanic
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18 potentiation (due to enhanced neurotransmitter release) and LTP in wild type hippocampal
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20 slices were unsuccessful in slices obtained from α 7 nAChR knockout mice. Thus, it appears that
21
22 endogenously produced oligomeric A β is capable of supporting hippocampal synaptic plasticity,
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24 learning and memory and utilizes α 7 nAChRs.
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33 From these sets of studies, A β interaction with α 7 nAChRs clearly has both positive and
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35 negative effects on neurotransmitter release, synaptic transmission, synaptic plasticity, learning
36
37 and memory. What decides one outcome over the other? The simplest answer to this question
38
39 is A β concentration in that picomolar concentrations of focally (acutely) delivered A β
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41 potentiates glutamatergic neurotransmission, synaptic potentiation, and enhanced learning and
42
43 memory. High concentrations (nanomolar- μ M) of acutely applied A β led to reduced synaptic
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45 NMDA receptors, reduced glutamatergic transmission and, presumably, impaired synaptic
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47 potentiation. Alternatively, A β -induced LTP impairment and cognitive deficits can be achieved
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49 with moderate concentrations of A β when it is delivered for an extended time period (300
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51 pmoles/day). Thus, as is the case with traditional nAChR agonists, acute exposure to moderate
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53 doses leads to receptor activation; exposure to high concentrations or prolonged exposure to
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4 moderate concentrations can lead to receptor inactivation through a desensitization
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7 mechanism.

9 **5.3 GLIAL α 7 nAChRs**

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11 An emerging issue in the field of α 7 nicotinic receptor research is to decipher the
12
13 functional role of these receptors on glial cells. Glial cells outnumber neurons in the brain and
14
15 their traditional designation as housekeeping cells continues to be reconsidered as
16
17 experimental observations indicate direct glial contributions synaptic function. Glial cells
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19 express functional receptors to many neurotransmitters and neuromodulators including,
20
21 glutamate, GABA, ATP and ACh and there is now evidence of α 7 nAChRs expression on most of
22
23 the major glial types: microglia, NG2 cells and astrocytes (97-100). The activation of α 7 nAChR
24
25 on cultured microglia reduces the release of a major inflammatory mediator in the CNS, TNF- α
26
27 (87). Given that TNF- α is abundantly found in the AD brain suggests that, under AD-like excess
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29 A β conditions, these receptors may be chronically inactivated through prolonged interaction
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31 with A β . Brain microglia and therefore brain inflammation are subject to α 7 nAChR regulation
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33 which has direct relevance to AD.
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43 Because astrocytes can release neurotransmitters to modulate neuronal excitability and
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45 synaptic transmission, there is much interest in the role of astrocytes in brain function. Since α 7
46
47 nAChRs flux Ca^{2+} , and changes in intracellular Ca^{2+} are the basis of astrocytic "excitability", the
48
49 activation of these receptors could be a potent mechanism for modulating astrocytic activity
50
51 (88). In cultured hippocampal astrocytes Sharma and Vijayaraghavan (86) found that focal ACh
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53 application induced inward currents in recorded astrocytes which were blocked by MLA. Ca^{2+}
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55 imaging experiments revealed intracellular Ca^{2+} elevations that persisted for tens of seconds,
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4 which were shown to be caused by an extracellular Ca^{2+} influx eliciting ryanodine receptor
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6 mediated CICR.
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9 Evidence of α 7 nAChR-mediated astrocyte functional responses in slice preparations is
10 scarce. However, we have shown astrocytic Ca^{2+} elevations in slice preparations of
11 hippocampus and neocortex elicited by focal nicotine and $\text{A}\beta_{1-42}$ (100 pM) application; these
12
13 experiments were performed in the presence of tetrodotoxin (TTX) suggesting that local
14
15 network activity was not responsible for the observed astrocytic Ca^{2+} elevations (Parri and
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17 Dineley, 2008a). The α 7 nAChRs competitive antagonist, MLA, blocked these responses.
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25 Additional evidence for functional α 7 nAChRs on glial cells comes from α 7 nAChR
26 mediated inward currents in area CA1 NG2 cells from hippocampal slices in response to
27 nicotine and (101). These currents were potentiated by the potent α 7 nAChR allosteric
28
29 modulator PNU-120596 and blocked by the α 7 nAChR selective antagonist MLA, but not DH β E,
30
31 an α 4 nAChR specific antagonist. These studies have significance because NG2 cells have been
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33 postulated to be glial precursors; therefore, $\text{A}\beta$ – α 7 nAChR interaction may differentially lead
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35 microglial versus astrocyte genesis.
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43 In summary, evidence is emerging that glial-resident α 7 nAChRs can functionally
44 contribute to NG2 cell development, modulate TNF- α production by microglia, and induce
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46 intracellular Ca^{2+} signaling in astrocytes that impinge upon neuronal synaptic signaling. In some
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48 cases, it is evident that $\text{A}\beta$ modulates these responses therefore implicating the $\text{A}\beta$ – α 7 nAChR
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50 interaction on glia in AD inflammation and possibly cognitive function.
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56 **6. nAChRs, $\text{A}\beta$, AND ALZHEIMER'S DISEASE**

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58 Understanding the molecular mechanism behind the selective vulnerability of
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60 cholinergic neurons to $\text{A}\beta$ toxicity would greatly advance our capabilities in treating AD. The
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4 fact that vulnerable neuron populations happen to be enriched for $\alpha 7$ nAChRs may provide an
5
6 important clue. As discussed previously, one possibility as A β accumulates during AD is that the
7
8 neuroprotective function of nAChR activation is blocked by the antagonizing effect of A β
9
10 peptides. Another possibility is that the A β – nAChR interaction under disease conditions
11
12 directly contributes to neurotoxicity. Potential mechanisms for each of these possibilities will
13
14 be discussed in the following sections.
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19 20 **6.1. nAChRs PROTECT AGAINST A β TOXICITY**

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22 *In vitro* studies utilizing cultured neurons have demonstrated that $\alpha 7$ nAChRs mediate,
23
24 at least in part, the neuroprotective effects of nicotine against A β toxicity (102). Protection
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26 against A β toxicity is proportional to the number of $\alpha 7$ nAChRs expressed by cultured cells
27
28 (103). Chronic exposure to A β ₁₋₄₂ *in vitro* leads to up-regulation of $\alpha 7$ nAChRs in a manner
29
30 similar to the effects of chronic nicotine treatment (50, 78). Tg2576 mice that produce
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32 excessive A β continue to up-regulate cortical and hippocampal $\alpha 7$ nAChRs as these animals age
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34 possibly providing an explanation as to why this AD model does not exhibit a cholinergic lesion
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36 phenotype nor significant loss of hippocampal and neocortical neurons (50, 78, 104, 105) (106,
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38 107).
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46 $\alpha 7$ nAChR-mediated neuroprotection against A β is via activation of the PI3K pathway;
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48 several lines of evidence suggest that this can occur through transactivation of src and tyrosine
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50 kinase receptors, including the high-affinity NGF receptor, TrkA (80, 108-111). Paradoxically, at
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52 low to moderate concentrations of soluble A β ₁₋₄₂, PI3K is also activated, suggesting that when
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54 A β is soluble and at non-disease concentration, the A β - nAChR interaction can lead to
55
56 activation of neurotrophic mechanisms (38). *In vitro* and *in vivo*, chronic nicotine leads to an
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58 increase in TrkA, in addition to $\alpha 7$ and $\alpha 4\beta 2$ nAChRs; *in vivo*, this is accompanied by up-
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4 regulation of ChAT and VAcHT in hippocampus (111-113). Increased TrkA is neuroprotective
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6 against A β toxicity; high concentrations of A β are neurotoxic and block nicotine-induced TrkA
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8 upregulation (110, 111). Thus, nAChRs are neuroprotective both by modulating the
9
10 neurotrophic system crucial for the maintenance of cholinergic neuron integrity as well as
11
12 stimulating signal transduction pathways that support neuron survival. Additionally, these
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14 studies suggest that in a situation of excess A β , nAChR function is blocked thus blocking its
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16 trophic activity and possibly contributing to A β toxicity. Taken together, one might imagine
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18 that under normal physiologic conditions, an A β – nAChR interaction provides a trophic signal;
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20 as A β accumulates, this interaction either blocks nAChR-mediated trophism or the A β – nAChR
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22 interaction under these circumstances becomes toxic.
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30 **6.2. MULTIPLE OPPORTUNITIES FOR AN $\alpha 7$ nAChR – A β INTERACTION TO CONTRIBUTE TO AD ETIOLOGY**

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33 Estimates of A β content in non-demented brain report picomolar values, however these
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35 estimates increase to nanomolar quantities for AD brain (46, 47, 83). Several studies report
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37 that prolonged exposure of nAChRs to nanomolar A β results in significant block of receptor
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39 function (54-58, 68). This suggests that under disease conditions an A β – nAChR interaction
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41 would interfere with the normal function of these receptors. Given the overwhelming evidence
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43 that nAChRs perform a neuroprotective role, an A β – nAChR interaction under elevated A β
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45 conditions may exacerbate the toxicity of A β by diminishing the neuroprotective signaling
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47 performed by these receptors. The current literature indicates that additional outcomes of an
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49 A β – nAChR interaction under ‘high A β ’ conditions could yield 1) perturbation and dysregulation
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51 of signal transduction mechanisms involved in synaptic plasticity and homeostasis; 2) receptor –
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53 peptide complex internalization; 3) cell toxicity; and 4) plaque seeding. The evidence for these
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55 mechanisms will be discussed in the following section.
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4 While there is a general consensus that the presence of excess A β is perhaps the most
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6 fundamental neurotoxic event in AD, several lines of evidence indicate that oligomeric, soluble
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8 forms of A β , rather than amyloid plaques, initiate the cognitive deficits characteristic of the
9
10 disease (114) (115). For example, transgenic mouse models for AD in which A β is over
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12 produced and accumulates in the CNS develop memory impairments long before plaques are
13
14 detected and in the absence of significant neuronal loss (78, 104, 105, 116). Furthermore,
15
16 introduction of A β oligomers produced *in vitro* or *in vivo* induces learning and memory deficits
17
18 in wildtype rodents that resemble those of transgenic models for AD (33, 34, 117). Therefore,
19
20 some of the cognitive impairments in AD may not be associated with extensive neuronal death;
21
22 rather, they may be the result of more subtle functional changes induced by soluble A β . It will
23
24 be important for future studies of the A β – nAChR interaction to attribute outcomes of this
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26 interaction not only to the concentration of soluble A β but also to specific structures and
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28 aggregates of the peptide.
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38 In addition to extracellular deposits of insoluble A β in plaques that are a primary
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40 histopathological diagnostic marker for AD, observations made as the 20th century yielded to
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42 the 21st, identified A β immunostaining within neurons and glia of post mortem AD samples
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44 (118, 119). Later, it was discovered that $\alpha 7$ nAChRs are not only expressed on neurons, this
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46 receptor is expressed by astrocytes and microglia (86, 87). Consequently, several groups have
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48 investigated the possibility that an $\alpha 7$ nAChR – A β interaction on these cell types are part of AD
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50 etiology.
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56 In a series of publications from Wang, Nagale, D'Andrea and colleagues, this group first
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58 explored the model that an $\alpha 7$ nAChR – A β interaction leads to intracellular accumulation of A β .
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60 Initial work utilizing post mortem AD brains and immunostaining approaches revealed that A β ₁.
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4 $A\beta_{1-42}$ was localized intracellularly in neurons and astrocytes of AD brains; neurons and astrocytes
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6 that had accumulated large amounts of $A\beta_{1-42}$ also highly expressed α 7 nAChRs (41, 120-123).
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9 In neuroblastoma cells transfected with α 7 nAChR cDNA, transfected cells exhibited rapid
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11 binding, internalization and accumulation of exogenous $A\beta_{1-42}$, but not $A\beta_{1-40}$; this
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13 internalization was related to the level of α 7 nAChR expression (41). Further, the α 7 nAChR
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15 antagonist, BTX, prevented $A\beta_{1-42}$ uptake. These results suggest that α 7 nAChRs facilitate $A\beta_{1-42}$
16
17 internalization and may confer selective vulnerability of specific cell types to the toxic effects of
18
19 intracellular $A\beta_{1-42}$. Nagale and colleagues took this notion a step further by suggesting that α 7
20
21 nAChR – $A\beta$ interaction and internalization may actually lead to plaque formation when the
22
23 host cell eventually dies and deposits the intracellular contents in the brain parenchyma (121,
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25 122, 124).
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33 The identification of α 7 nAChR and $A\beta$ within astrocytes also provided the first
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35 indication that nAChR – $A\beta$ interaction may be an important event in the inflammatory
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37 progression of the disease (121). These studies showed that $A\beta_{1-42}$ and α 7 nAChR proteins were
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39 co-localized in intensely GFAP-positive (activated) astrocytes in immunostained AD brain. Since
40
41 these studies also identified ChAT, the authors proposed a model in which $A\beta$ and α 7 proteins
42
43 are phagocytosed by activated astrocytes in the vicinity of neuronal remnants. As neuronal
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45 debris accumulates in the astrocyte, astrocyte viability is compromised and eventually kills the
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47 cell leaving behind $A\beta$ deposits rich in astrocytic GFAP, and neuronal markers such as ChAT and
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49 α 7 protein.
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56 A slightly different interpretation was made by Teaktong *et al.* (125) when they found
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58 that the majority of astrocytes in AD hippocampus and cortex also *express* α 7 nAChRs; this
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60 group deduced that α 7 nAChRs are up-regulated on astrocytes in AD. Follow-up studies
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4 determined that the number of astrocytes double-labeled with α 7 nAChR and GFAP antibodies
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6 was increased in most areas of the hippocampus and entorhinal cortex in AD compared with
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8 controls suggesting that increased astrocyte alpha7 nAChRs in AD may be associated with
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10 inflammatory mechanisms related to degenerative processes (126).
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14 Although the work by Teaktong et al. and Nagale et al. utilized antibodies to α 7 nAChRs
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16 that have since come into question as to their specificity, subsequent work from Dr. Agneta
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18 Nordberg's group using 125 I-BTX to quantify α 7 nAChR protein supports these initial
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20 observations both in post mortem AD brain and primary cell culture (127-129). Subsequent *in*
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22 *vitro* work by Xiu *et al.* (129) lends support to the idea that A β -induced α 7 nAChR up-regulation
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24 on astrocytes occurs in the disease: exposure of cultured primary astrocytes to picomolar –
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26 nanomolar A β ₁₋₄₂ for 48 hours followed by quantification of mRNA and protein with RT-PCR and
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28 immunoblot, respectively, resulted in up-regulation of both α 7 nAChR mRNA and protein.
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34 A recent study utilizing cultured rat microglia found that activation of microglial α 7
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36 nAChR leads to increased A β phagocytosis (130). The study found that human A β ₁₋₄₂ clearance
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38 was increased by nicotine as well as the cholinesterase inhibitor and α 7 nAChR allosteric
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40 modulator galantamine; A β clearance was blocked by the broad nAChR antagonist MEC and the
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42 α 7 nAChR selective antagonist MLA, but not atropine a mAChR antagonist. Furthermore,
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44 galantamine-treated AD mice exhibited reduced amyloid load as did rats that received intra-
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46 hippocampal injections of human A β ₁₋₄₂. Thus, these studies suggest that microglia-resident α 7
47
48 nAChRs may be part of the mechanism mediating the therapeutic efficacy of this compound
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50 through A β clearance.
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58 The interaction of α 7 nAChRs on astrocytes with A β peptide may provide a possible link
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60 between α 7 nAChRs and the inflammatory processes of AD. Analogous to its role in the
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4 periphery, $\alpha 7$ nAChR activation on glia shunts TNF- α production and release; therefore, an
5
6 important question to answer is under what conditions might an $\alpha 7$ nAChR – A β interaction
7
8 lead to decreased TNF- α production as opposed to general microglial and astrocyte activation.
9
10 It will be important to decipher this apparently complex relationship between neuroprotection,
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12 astrocyte activation, inflammation, and neurotoxicity, in addition to how this interaction
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14 contributes to the development of neuronal- and astrocyte-derived plaques in AD brain.
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Clearly, an interaction between glial-resident $\alpha 7$ nAChRs and A β may be involved in a broad array of outcomes during the progression of AD.

We recently tested the hypothesis that $\alpha 7$ nAChRs are neuroprotective during early stage AD by investigating the effects of $\alpha 7$ nAChR gene deletion on cognitive function and septo-hippocampal integrity in the Tg2576 APP transgenic animal model for AD (131). Whereas $\alpha 7$ nAChR knock-out (A7KO) mice neither show cognitive deficits nor exhibit morphological CNS abnormalities, we found that cognitive deficits seen in 5-months-old APP transgenic mice are more severe when $\alpha 7$ nAChR receptors are absent (A7KO-APP) (93, 131, 132). Biochemical analyses on 5-months-old A7KO-APP revealed significant reduction in hippocampal and basal forebrain ChAT activity and loss of hippocampal neurons and markers compared to APP mice. Consistent with lesion studies and observations in AD brain, compromise of basal forebrain cholinergic function leads to similar concessions within the hippocampus of 5-months-old A7KO-APP mice. These studies demonstrated that $\alpha 7$ nAChRs mediate neuroprotective mechanisms that maintain the basal forebrain cholinergic phenotype and preserve hippocampal integrity; loss of basal forebrain cholinergic integrity is accelerated and exacerbated when $\alpha 7$ nAChRs are absent and misfolded A β is in excess.

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4 Data continues to accumulate demonstrating that A β peptides interact with α 7 nAChRs
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6 with especially high affinity and for extended periods of time; consistent with such an
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8 interaction, up-regulation of α 7 nAChRs mRNA and protein has been reported in astrocytes,
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10 peripheral blood leukocytes and cortical and hippocampal neurons harvested from the tissue of
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12 AD patients (10, 125, 133). The additional observation that, in early AD, A β preferentially
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14 accumulates in neuronal populations that are enriched for α 7 nAChRs may be one reason for
15
16 the selective vulnerability of the basal forebrain cholinergic system to A β toxicity.
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22 In one study, mRNA expression levels of nicotinic and muscarinic AChR subtypes and
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24 ChAT were measured in single cells isolated from the cholinergic basal forebrain of post-
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26 mortem AD tissue (and non-cognitively impaired controls) then individually analyzed using
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28 microarray methods. No differences in mRNA expression were observed for the other nAChR
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30 subunits, mAChR subtypes or ChAT (16). However, cells from AD basal forebrain exhibited a
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32 significant up-regulation of α 7 nAChR subunit mRNAs (16). This increase in α 7 nAChR
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34 expression levels within CBF neurons was inversely correlated with Global Cognitive Score and
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36 with Mini-Mental State Examination performance (16). We would posit that these increases in
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38 α 7 nAChR protein within the basal forebrain result from direct interaction with A β and receptor
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40 desensitization followed by receptor up-regulation (66). In fact, α 7 nAChR-selective agonists
41
42 are unable to activate these receptors in APP transgenic mice and recent work on human AD
43
44 postmortem brain samples indicate that much of the receptor protein is functionally
45
46 inactivated due to association with A β peptide (9, 10, 134, 135). In addition, recent studies
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48 have shown that these A β - α 7 nAChR protein complexes occur primarily in brain regions
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50 targeted by the cholinergic basal forebrain; disruption of this association in post-mortem AD
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52 cortex leads to increased availability of functional α 7 nAChRs (10). These observations suggest
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4 that in AD, $\alpha 7$ nAChRs are likely inactive due to desensitization as a consequence of prolonged
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6 association with A β peptide.
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10 Based on current understanding, we propose that soluble A β oligomers that may lead to
11
12 the transient activation of $\alpha 7$ nAChRs and subsequent initiation of both neuroprotective and
13
14 neurotrophic signaling mechanisms that have been elucidated *in vitro* (38, 50, 80). An
15
16 additional benefit may be provided via sequestering A β oligomers and preventing further
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18 oligomerization, thus deviating A β from additional toxic interactions (e.g., mediators of
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20 glutamatergic neurotransmission; (136, 137). As AD progresses, we envision that A β
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22 accumulates and irreversibly associates in a manner that overwhelms the availability of $\alpha 7$
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24 nAChRs leading to functional blockade and loss of neuroprotective signaling.
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30 **6.3. THERAPEUTIC OPPORTUNITIES**

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33 While much progress has been made regarding the nature of $\alpha 7$ nAChR – A β interaction
34
35 *in vivo* and *in vitro*, many questions remain as to the exact features of $\alpha 7$ nAChR – A β
36
37 interaction during the initiation and progression of AD to confidently suggest viable therapeutic
38
39 strategies. Nonetheless, one might consider a few possibilities based on the extant literature
40
41 reviewed here. Several $\alpha 7$ nAChR agonists and positive allosteric modulators (PAMs) have been
42
43 developed as therapeutic agents targeting central and peripheral disorders that involve pain,
44
45 inflammation, schizophrenia, and AD (138-140). One such compound, S-24795, was recently
46
47 directly tested for efficacy in AD; application to homogenates prepared from post mortem AD
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49 brain was found to facilitate A β dissociation from the receptor in order to resurrect $\alpha 7$ nAChR
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51 function and its neuroprotective properties (10, 42). Possibly, S-24795, and other such $\alpha 7$
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53 nAChR PAMs would prove beneficial during early AD by both inhibiting and partially reversing
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55 A β binding to $\alpha 7$ nAChRs. However it remains to be seen if the dislodged A β is then free to
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4 interact in alternative yet deleterious ways. Possibly coincident A β immunotherapy would
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6 alleviate this potential negative side effect of $\alpha 7$ nAChR PAM therapy. Another possible
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8 strategy, albeit somewhat difficult to envision at the receptor level, would be to develop a
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10 compound that is capable of maintaining $\alpha 7$ nAChR neuroprotective signaling capabilities on
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12 the one hand and continue to sequester A β on the other. Again, this in conjunction with
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14 interventions that decrease oligomeric A β levels might prove most efficacious.
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20 Assuming that, under normal physiological conditions, A β and $\alpha 7$ nAChRs interact and
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22 result in receptor activation implies that this interaction may serve a neuroprotective role given
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24 that $\alpha 7$ nAChRs couple to neuroprotective signaling cascades (PI3K etc). Therefore, it seems
25
26 imprudent to prophylactically block all $\alpha 7$ nAChR – A β interaction. However, as AD progresses
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28 and soluble A β acquires pathological concentrations and conformations, it might be useful to
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30 develop ways in which to interrupt specific $\alpha 7$ nAChR – A β interactions, especially if this
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32 interaction antagonizes receptor function or is involved in accumulating intracellular A β . As is
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34 being currently pursued, targeting A β directly with immunotherapy is one approach.
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40 From the nAChR side of the equation, the development of a decoy nAChR-like binding
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42 site (presuming it is known) could prevent toxic $\alpha 7$ nAChR – A β interactions. Direct modulation
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44 of nAChR function is another strategy. However this requires a solid understanding of the
45
46 functional relationship between the receptor and peptide as A β levels increase and different
47
48 conformations of the peptide accumulate with disease progression. Clearly, while great strides
49
50 have been made in understanding the $\alpha 7$ nAChR – A β interaction in recent years, the likely
51
52 complex nature of this relationship as AD progresses and soluble A β acquires additional
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54 aggregation conformers demands that much has yet to be understood before emphatically
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56 stating what the best $\alpha 7$ nAChR therapeutic strategy is for AD.
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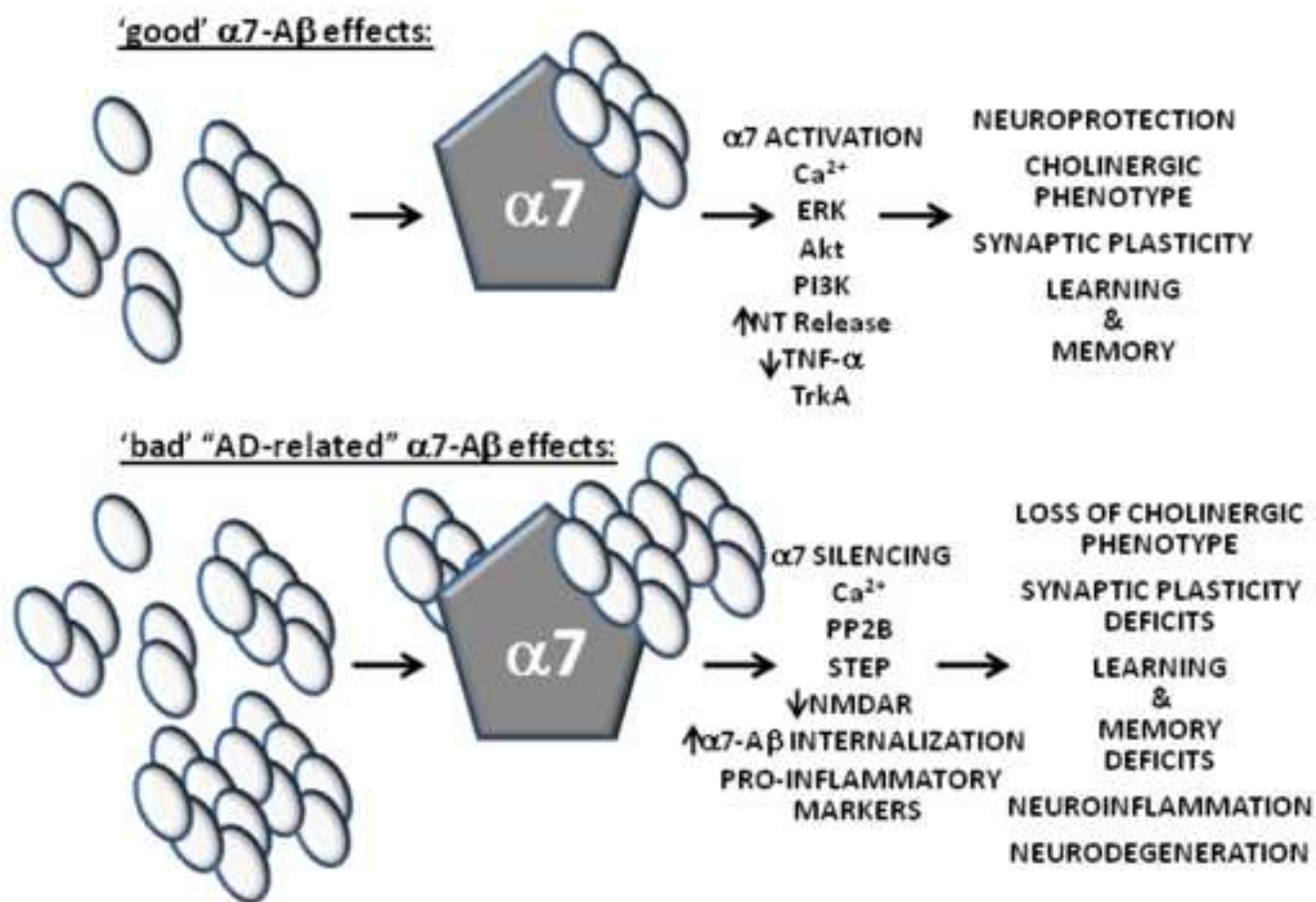
FIGURE LEGENDS

Figure 1. Summary of the signal transduction consequences of $A\beta$ activation of $\alpha 7$ nAChRs.

$A\beta_{1-42}$ acting through $\alpha 7$ nAChRs activates PKA downstream of ERK MAPK PI3K (and Akt) is an intermediary between $\alpha 7$ nAChR activation and ERK MAPK phosphorylation since LY294002 blocked ERK MAPK activation following $A\beta_{1-42}$. ERK MAPK activation leads to p90 Rsk and CREB phosphorylation; $A\beta_{1-42}$ -induced p90 Rsk phosphorylation is carried out by both ERK and PKA since U0126 completely obliterated p90 Rsk phosphorylation and H-89 partially reduced it.

Adapted from [43, 55, 64, 81].

Graphical Abstract



nAChR Subtype	Experimental Preparation	Type of Interaction	Possible Mechanism/ Downstream Consequence	Reference
Human $\alpha 7$	Human Brain: Control & AD	Co-Localization & Co-IP	High-Affinity Interaction	32
Human $\alpha 7$	Cell Culture	High-Affinity Binding	Competitive Binding (BTX, A β)	33
Human $\alpha 7$	<i>Xenopus</i> Oocytes	Functional Antagonism (reversible)	Noncompetitive (ACh)	63
Human $\alpha 7$	<i>Xenopus</i> Oocytes	Functional Antagonism (reversible)	Noncompetitive (ACh)	60
Human $\alpha 7$	SY5Y Neuroblastoma Cells	Receptor Activation	ERK MAPK Activation	44
Rat $\alpha 7$, non- $\alpha 7$	Acute Hippocampal Slice, GABAergic Interneurons	Functional Antagonism (reversible)	Decreased Open p_o	72
Rat $\alpha 7$	Cultured Hippocampal Neurons	Functional Antagonism (reversible)	Noncompetitive (ACh)	75
Rat $\alpha 7$	<i>Xenopus</i> Oocytes	Receptor Activation	Ca ²⁺ Influx	55, 59
Rat $\alpha 7$	<i>Xenopus</i> Oocytes	No effect	N/A	62
Rat, Mouse $\alpha 7$	Isolated Presynaptic Terminals	Receptor Activation	Increased Presynaptic [Ca ²⁺] _i	57, 58
Mouse $\alpha 7$	Cultured Hippocampal & Cortical Neurons	Receptor Activation	Akt Phosphorylation	81
Rat, Mouse $\alpha 7\beta 2$	Acute Basal Forebrain Slice, <i>Xenopus</i> Oocytes,	Receptor Antagonism	Reversible	75
$\alpha 7$ Knock-out Mice	Isolated Presynaptic Terminals	Absence of Receptor Activation	Loss of Increased Presynaptic [Ca ²⁺] _i	77

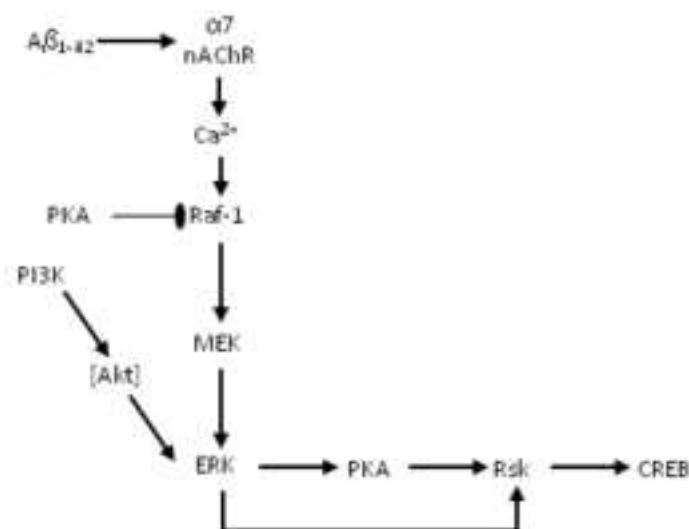


Figure 1. Summary of the signal transduction consequences of Aβ₁₋₄₂ activation of α7 nAChRs. Aβ₁₋₄₂ acting through α7 nAChRs activates PKA downstream of ERK MAPK. PI3K is an intermediary between α7 nAChR activation and ERK MAPK phosphorylation since LY294002 blocked ERK MAPK activation following Aβ₁₋₄₂. ERK MAPK activation leads to p90 Rsk and CREB phosphorylation; Aβ₁₋₄₂-induced p90 Rsk phosphorylation is carried out by both ERK and PKA since U0126 completely obliterated p90 Rsk phosphorylation and H-89 partially reduced it. Adapted from [43, 55, 64, 81].