Manuscript Details

Manuscript number	YPHRS_2018_1130_R1
Title	BETA-AMYLOID SHORT- AND LONG-TERM SYNAPTIC ENTANGLEMENT
Article type	Review Article

Abstract

Beta-amyloid (Aβ) is a peptide that derives from the proteolytic cleavage of the amyloid precursor protein (APP) by several secretases. Since its isolation and sequencing from Alzheimer's disease (AD) brains, Aβ has been intensively investigated in the context of AD as the main pathogenic marker responsible for neurodegenerative processes. During the last three decades, results from several independent studies have converged to form the so-called amyloid cascade hypothesis of AD and several therapeutic strategies designed to modulate the APP amyloidogenic pathway have been developed. However, none of the clinical trials targeting Aβ culminated in a significant clinical outcome, thus challenging the concept that targeting Aβ, at least within the time window so far explored in clinical trials, may have a therapeutic effect. However, besides its presence in AD brains, brain cells produce Aβ, thus suggesting that, under normal conditions, the peptide may have a role in the regulation of brain functions, which is consistent with its ubiquitous presence and normal synthesis. Taking into account that Aβ has been found to exhibit a dual role strictly correlated with its concentration (neuromodulatory/neuroprotective vs neurotoxic), we discuss emerging evidence indicating that physiological concentrations of Aβ peptide modulate synaptic activity. The review examines the physiological effects of Aβ on acute synaptic activities and the functional interplay existing between Aβ and different neurotransmitter systems, i.e. cholinergic, glutamatergic, GABAergic, catecholaminergic, serotoninergic, and peptidergic. The review also provides an insight into the different mechanisms through which Aβ affects synaptic activity, focusing in particular on Aβ interaction with the key synaptic proteins that regulate the neurotransmitter release machinery. These interactions may help to identify or recognize alterations in neurotransmitter activity and correlated behaviors as predictive signs for the development of AD and to understand the limitations of current interventions and the failure so far of amyloid targeted therapies.

Keywords	beta-amyloid, acute synaptic activity, neurotransmitter release, SNARE complex, behavioral correlates

Corresponding Author	Stefano Govoni
Corresponding Author's Institution	University of Pavia
Order of Authors	Cristina Lanni, Francesca Fagiani, Marco Racchi, Stefania Preda, Alessia Pascale, Massimo Grilli, Nicola Allegri, Stefano Govoni

Suggested reviewers	Agata Copani, Jeffrey Cummings, ottavio arancio

Submission Files Included in this PDF

File Name [File Type]

Lanni et al_Cover letter_revision.pdf [Cover Letter]

Lanni et al_response reviewers.docx [Response to Reviewers]

Graphical abstract.tif [Graphical Abstract]

Lanni et al_manuscript_Revision.pdf [Manuscript File]

Figure 1.tif [Figure]

Figure 2.tif [Figure]

Lanni et al_Table 1_revision.pdf [Table]

Lanni et al_Table 2_revision.pdf [Table]

Lanni et al_Table 3_revision.docx.pdf [Table]

Author declaration_Pharmacol Review Ab.pdf [Conflict of Interest]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
No data was used for the research described in the article

Dipartimento di Scienze del Farmaco
Università degli Studi di Pavia

Prof E. Clementi,
Editor-in-Chief
Università degli Studi di Milano,
Milano, Italy

RE: Manuscript YPHRS_2018_1130

Dear Prof. Clementi,

Please find enclosed the revised manuscript entitled: "BETA-AMYLOID SHORT- AND LONG-TERM SYNAPTIC ENTANGLEMENT", authored by Lanni C, Fagiani F, Racchi M, Preda S, Pascale A, Grilli M, Allegri N, Govoni S, which is under consideration for publication as Review Article to Pharmacological Research.

As you suggested in your e-mail on 11th of September 2018, we have performed the modifications requested by the Reviewers and we have made appropriate changes throughout the manuscript. The latter are outlined in the enclosed detailed response to the Reviewers.

We wish to thank the Reviewers as we think that the referee's comments allowed us to greatly improve the paper and we hope that the revised version will now be suitable for publication in Pharmacological Research.

We hereby state that this article is original, is not under consideration, and has not been previously published elsewhere, either in whole or in part. The content of this article has not been anticipated by any previous publications and has not been posted on the Web site. All indicated authors have seen and approved the manuscript's content.

Sincerely yours,

Stefano Govoni, Ph.D.
Dept. of Drug Sciences - Pharmacology Section
University of Pavia
V.le Taramelli, 14
phone: ++39 0382987394
cell phone ++39 3493445141
e-mail: govonis@unipv.it

Response to Reviewers

Reviewer #1

We thank the Reviewer for the overall positive comment.

1) According to the Reviewer's suggestions, we expanded the section on the regulation of SNARE proteins by Aβ, mainly focusing on the direct interaction between the peptide and syntaxin 1A (Yang et al, Cell Rep. 2015;12(8):1244-51) and synaptophysin (Russell et al., PLoS One. 2012;7(8):e43201) (page 26, lanes 5-26).

2) As suggested by the Reviewer, beta-amyloid and alpha-synuclein are the components of AD and Parkinson's disease (PD) and dementia with Lewy bodies (DLB), respectively. We examined the effects of alpha-synuclein on synaptic pathology. Indeed, there are common points of interest with beta-amyloid-induced pathology. The aggregations of Aβ and alpha-synuclein are used as the major pathological markers of AD and PD (Irwin DJ, et al. Nat Rev Neurosci. 2013; 14(9):626-36). Aβ deposition has been found in patients with DLB (Armstrong RA, et al. Neurosci Lett. 1997; 227(3):193-6) and nearly half of AD patients have LB pathology (Hamilton RL. Brain Pathol. 2000; 10(3):378-84). Furthermore, familial AD mutations showed increased levels of LB pathology (Leverenz JB, et al. Arch Neurol. 2006; 63(3):370-6). These parallelisms suggest a substantial connection between AD and PD pathologies. Within this context, high concentrations of Aβ promoted the accumulation of alpha-synuclein and accelerated motor and memory deficits and cognitive dysfunction in transgenic mouse models. In addition, both Aβ and alpha-synuclein showed a dual role depending on their concentrations on the vesicle fusion thus influencing neurotransmitter release (Hou C, et al. Front Mol Neurosci. 2017;10:66; Lou X et al. Biochem J. 2017;474(12):2039-2049). Complexively, the studies of neurotransmitter release contribute significantly to clarify the pathogenesis of neurodegenerative diseases in brain, thus helping to illuminate the underlying pathogenic mechanisms for neurodegenerative disorders. These data are noteworthy since some patients have clinical and pathological features of both diseases, raising the possibility of overlapping pathogenic pathways and allowing the identification of subsets of patients. On the other hand, we have the impression that this discussion, even if of interest, might bring out of focus the review and we decided not to include it in the final text.

3) The text has been carefully revised for the English language.

Reviewer #2

We thank the Reviewer for the comments and the suggestions, that allowed us to greatly improve the paper.

Regarding the major concerns, the Reviewer is right and we have modified and/or integrated the text as follows:

1) We have rewritten some parts of the Introduction to make it less confusing. In particular:
   - we deepened the concept on the therapeutic strategies, referring to humanized antibodies against Aβ peptides, beta and gamma secretase inhibitors, and we better explained the reasons of the failure of these strategies (page 4, lanes 4-26).
   - Concerning the failure of the therapeutic strategies targeting beta amyloid, we rephreased the concept on the beta-amyloid theory: "The validity of the amyloid cascade hypothesis and the contribution of Aβ peptides to the progression of the disease cannot be discounted" (page 4, lanes 12-13).
   - As requested, we added the references "Puzzo et al, 2008" and “Puzzo and Arancio,

2013", when explaining that the dual role of Aβ peptides (neuromodulatory/neuroprotective vs neurotoxic) is strictly correlated with its concentration and aggregation state (page 4, lane 24-26, references 2-3).

- To avoid confusion when defining the focus of the review, we better specified that we have analyzed the effects of externally added low concentrations of beta amyloid on synaptic activity and the functional interplay with different neurotransmitter systems (page 5, lanes 1-4).

2) The Reviewer is right about our error on the areas with the highest deposits of beta amyloid. We rewrote the text as follows: "Such results are consistent with the hypothesis that neuronal activity regulates the regional vulnerability to Aβ deposition. Brain areas with high baseline levels of synaptic activity have been found to be the more prone to Aβ accumulation [Bero et al., 2011]. In particular, several studies investigating the impact of neuronal activity on Aβ levels has focused on the default mode network. By using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) with PIB compound, Buckner et al. reported a spatial overlap between the topography of amyloid deposition and the regions of the default mode network (e.g. posterior cingulate and parietal cortex, medial temporal lobe and medial frontal subsystem) [Buckner et al., 2005]. However, not all regions displaying high baseline activity are subject to Aβ accumulation and the relationship between neuronal activity and Aβ deposition cannot be considered linear. In a further study by Buckner et al., the strong network connectivity rather than elevated baseline activity has been correlated to regional Aβ deposition. Cortical regions (e.g. posterior cingulate, lateral temporal, lateral parietal, and medial/lateral prefrontal cortices) with intense interconnectivity have been found to display high Aβ deposition [Buckner et al., 2009]" (page 5, lanes 25-33 and page 6, lanes 1-6).

3) According to the Reviewer's suggestions, we have added the references from postmortem human brain studies. In particular,

- we have added the references "Davies and Maloney, 1976", "Perry et al., 1978", "Francis et al., 1985" when referring to the dysfunctional cholinergic transmission (page 9, lane 17, references 25-27).
- In regard of the stereological counts in the locus coeruleus in AD brains, we have included the appropriate reference (Wilson et al., 2013) as suggested by the reviewer (page 16, lane 2, reference 64).
- Concerning the alteration of SNARE machinery in AD, we added some of the suggested references and extended this section (page 25, lanes 29-33, page 26, lanes 1-30).

4) As suggested, we revised the references in the text and maintained preferentially original papers as source for the findings described in the review.

5) We agree with the comment of the Reviewer concerning the absence of a glutamatergic and GABAergic section in the neurochemistry studies. We added in the text the two sections, Glutamatergic system (pages 12-13) and GABAergic system (pages 14-15).

6) As suggested by the Reviewer, in the serotoninergic section we discussed the role of 5-HT6R antagonists in the AD drug development pipeline and reported recent data on the failure of the 5-HT6R antagonist idalopirdine, combined to the treatment with cholinesterase inhibitors, to improve cognitive performance or mitigate the cognitive decline (page 22, lanes 30-33, page 23, lanes 1-15).

7) Concerning the comment of a discussion on the antimicrobial function of Aβ peptide, in this review we specifically analyzed the modulation of synaptic activity and neurotransmission by Aβ peptides, when analyzing the physiological role of the peptide. We are aware that Aβ in human physiology may contribute to antimicrobial activity, but we think that a discussion

in the text on this point will be out of focus. Indeed, data from literature reported that Aβ not only binds and intercepts microbial pathogens, but also possesses antibacterial, antifungal, and antiviral properties against at least eleven species of microbes (Gosztyla ML et al., 2018 J Alzheimers Dis. 62(4):1495-1506). Furthemore, suggestions of an interaction between plaques formation and anti-microbial activity come from several studies demonstrating the presence of a variety of microbial species in Aβ plaques or AD brains. Specifically, HSV1 DNA (Wozniak MA, et al. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. J. Pathol. 217, 131-138; 2009), and *Borrelia* antigen and DNA (Miklossy J. Bacterial Amyloid and DNA are important constituents of senile plaques: further evidence of the spirochetal and biofilm nature of senile plaques. J. Alzheimer's Dis. 53, 1459-1473, 2016) have been found in plaque cores, and extracellular and intracellular *C. pneumoniae* and fungal infections have been reported in AD brain tissue (Hammond CJ, Hallock LR et al. Immunohistological detection of Chlamydia pneumoniae in the Alzheimer's disease brain. BMC Neurosci. 11:121, 2010; Pisa et al. Direct visualization of fungal infection in brains from patients with Alzheimer's disease. J. Alzheimer's Dis. 43, 613-624, 2015). These observations can be linked to the hypothesis that a viral infection may contribute to intraneuronal accumulation of Aβ, resulting in cell lysis, and the release of dense-core plaques containing viral DNA into the extracellular space, whereas an extracellular Aβ deposition may be due to the interception of bacterial or fungal pathogens (D'Andrea M. R. Bursting Neurons and Fading Memories: An Alternative Hypothesis of the Pathogenesis of Alzheimer's Disease, 1st Edn. New York, NY: Academic Press, 2014). As above stated, we decided not to include this discussion in the paper.

Regarding the minor comments:

1) The text has been carefully revised for the English language.

2) We made uniform "beta-amyloid" in the paper.

3) We corrected in the abstract section the sentence on beta-amyloid describing it as a peptide.

4) The Reviewer is right and we have provided full word of BACE (β-site APP-cleaving enzyme) in its first appearance in the text (page 6, lane 18).

5) Concerning the crosstalk between serotoninergic transmission and beta-amyloid related to depression and dementia, an explanation is present at page 29, lane 33, page 30, lanes 1-14).

UNIVERSITÀ DI PAVIA
Dipartimento di Scienze del Farmaco
Università degli Studi di Pavia

Response to Reviewers

Reviewer #1

We thank the Reviewer for the overall positive comment.

1) According to the Reviewer's suggestions, we expanded the section on the regulation of SNARE proteins by Aβ, mainly focusing on the direct interaction between the peptide and syntaxin 1A (Yang et al, Cell Rep. 2015;12(8):1244-51) and synaptophysin (Russell et al., PLoS One. 2012;7(8):e43201) (page 26, lanes 5-26).

2) As suggested by the Reviewer, beta-amyloid and alpha-synuclein are the components of AD and Parkinson's disease (PD) and dementia with Lewy bodies (DLB), respectively. We examined the effects of alpha-synuclein on synaptic pathology. Indeed, there are common points of interest with beta-amyloid-induced pathology. The aggregations of Aβ and alpha-synuclein are used as the major pathological markers of AD and PD (Irwin DJ, et al. Nat Rev Neurosci. 2013; 14(9):626-36). Aβ deposition has been found in patients with DLB (Armstrong RA, et al. Neurosci Lett. 1997; 227(3):193-6) and nearly half of AD patients have LB pathology (Hamilton RL. Brain Pathol. 2000; 10(3):378-84). Furthermore, familial AD mutations showed increased levels of LB pathology (Leverenz JB, et al. Arch Neurol. 2006; 63(3):370-6). These parallelisms suggest a substantial connection between AD and PD pathologies. Within this context, high concentrations of Aβ promoted the accumulation of alpha-synuclein and accelerated motor and memory deficits and cognitive dysfunction in transgenic mouse models. In addition, both Aβ and alpha-synuclein showed a dual role depending on their concentrations on the vesicle fusion thus influencing neurotransmitter release (Hou C, et al. Front Mol Neurosci. 2017;10:66; Lou X et al. Biochem J. 2017;474(12):2039-2049). Complexively, the studies of neurotransmitter release contribute significantly to clarify the pathogenesis of neurodegenerative diseases in brain, thus helping to illuminate the underlying pathogenic mechanisms for neurodegenerative disorders. These data are noteworthy since some patients have clinical and pathological features of both diseases, raising the possibility of overlapping pathogenic pathways and allowing the identification of subsets of patients. On the other hand, we have the impression that this discussion, even if of interest, might bring out of focus the review and we decided not to include it in the final text.

3) The text has been carefully revised for the English language.

Reviewer #2

We thank the Reviewer for the comments and the suggestions, that allowed us to greatly improve the paper.

Regarding the major concerns, the Reviewer is right and we have modified and/or integrated the text as follows:

1) We have rewritten some parts of the Introduction to make it less confusing. In particular:
   - we deepened the concept on the therapeutic strategies, referring to humanized antibodies against Aβ peptides, beta and gamma secretase inhibitors, and we better explained the reasons of the failure of these strategies (page 4, lanes 4-26).
   - Concerning the failure of the therapeutic strategies targeting beta amyloid, we rephreased the concept on the beta-amyloid theory: "The validity of the amyloid cascade hypothesis and the contribution of Aβ peptides to the progression of the disease cannot be discounted" (page 4, lanes 12-13).
   - As requested, we added the references "Puzzo et al, 2008" and "Puzzo and Arancio, 2013", when explaining that the dual role of Aβ peptides (neuromodulatory/neuroprotective vs neurotoxic) is strictly correlated with its concentration and aggregation state (page 4, lane 24-26, references 2-3).
   - To avoid confusion when defining the focus of the review, we better specified that we have analyzed the effects of externally added low concentrations of beta amyloid on synaptic activity and the functional interplay with different neurotransmitter systems (page 5, lanes 1-4).

2) The Reviewer is right about our error on the areas with the highest deposits of beta amyloid. We rewrote the text as follows: "Such results are consistent with the hypothesis that neuronal activity regulates the regional vulnerability to Aβ deposition. Brain areas with high baseline levels of synaptic activity have been found to be the more prone to Aβ accumulation [Bero et al., 2011]. In particular, several studies investigating the impact of neuronal activity on Aβ levels has focused on the default mode network. By using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) with PIB compound, Buckner et al. reported a spatial overlap between the topography of amyloid deposition and the regions of the default mode network (e.g. posterior cingulate and parietal cortex, medial temporal lobe and medial frontal subsystem) [Buckner et al., 2005]. However, not all regions displaying high baseline activity are subject to Aβ accumulation and the relationship between neuronal activity and Aβ deposition cannot be considered linear. In a further study by Buckner et al., the strong network connectivity rather than elevated baseline activity has been correlated to regional Aβ deposition. Cortical regions (e.g. posterior cingulate, lateral temporal, lateral parietal, and medial/lateral prefrontal cortices) with intense interconnectivity have been found to display high Aβ deposition [Buckner et al., 2009]" (page 5, lanes 25-33 and page 6, lanes 1-6).

3) According to the Reviewer's suggestions, we have added the references from postmortem human brain studies. In particular,
   - we have added the references "Davies and Maloney, 1976", "Perry et al., 1978", "Francis et al., 1985" when referring to the dysfunctional cholinergic transmission (page 9, lane 17, references 25-27).
   - In regard of the stereological counts in the locus coeruleus in AD brains, we have included the appropriate reference (Wilson et al., 2013) as suggested by the reviewer (page 16, lane 2, reference 64).
   - Concerning the alteration of SNARE machinery in AD, we added some of the suggested references and extended this section (page 25, lanes 29-33, page 26, lanes 1-30).

4) As suggested, we revised the references in the text and maintained preferentially original papers as source for the findings described in the review.

5) We agree with the comment of the Reviewer concerning the absence of a glutamatergic and GABAergic section in the neurochemistry studies. We added in the text the two sections, Glutamatergic system (pages 12-13) and GABAergic system (pages 14-15).

6) As suggested by the Reviewer, in the serotoninergic section we discussed the role of 5-HT6R antagonists in the AD drug development pipeline and reported recent data on the failure of the 5-HT6R antagonist idalopirdine, combined to the treatment with cholinesterase inhibitors, to improve cognitive performance or mitigate the cognitive decline (page 22, lanes 30-33, page 23, lanes 1-15).

7) Concerning the comment of a discussion on the antimicrobial function of Aβ peptide, in this review we specifically analyzed the modulation of synaptic activity and neurotransmission by Aβ peptides, when analyzing the physiological role of the peptide. We are aware that Aβ in human physiology may contribute to antimicrobial activity, but we think that a discussion in the text on this point will be out of focus. Indeed, data from literature reported that Aβ not only binds and intercepts microbial pathogens, but also possesses antibacterial, antifungal, and antiviral properties against at least eleven species of microbes (Gosztyla ML et al., 2018 J Alzheimers Dis. 62(4):1495-1506). Furthemore, suggestions of an interaction between plaques formation and anti-microbial activity come from several studies demonstrating the presence of a variety of microbial species in Aβ plaques or AD brains. Specifically, HSV1 DNA (Wozniak MA, et al. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. J. Pathol. 217, 131-138; 2009), and *Borrelia* antigen and DNA (Miklossy J. Bacterial Amyloid and DNA are important constituents of senile plaques: further evidence of the spirochetal and biofilm nature of senile plaques. J. Alzheimer's Dis. 53, 1459-1473, 2016) have been found in plaque cores, and extracellular and intracellular *C. pneumoniae* and fungal infections have been reported in AD brain tissue (Hammond CJ, Hallock LR et al. Immunohistological detection of Chlamydia pneumoniae in the Alzheimer's disease brain. BMC Neurosci. 11:121, 2010; Pisa et al. Direct visualization of fungal infection in brains from patients with Alzheimer's disease. J. Alzheimer's Dis. 43, 613-624, 2015). These observations can be linked to the hypothesis that a viral infection may contribute to intraneuronal accumulation of Aβ, resulting in cell lysis, and the release of dense-core plaques containing viral DNA into the extracellular space, whereas an extracellular Aβ deposition may be due to the interception of bacterial or fungal pathogens (D'Andrea M. R. Bursting Neurons and Fading Memories: An Alternative Hypothesis of the Pathogenesis of Alzheimer's Disease, 1st Edn. New York, NY: Academic Press, 2014). As above stated, we decided not to include this discussion in the paper.

Regarding the <u>minor comments</u>:

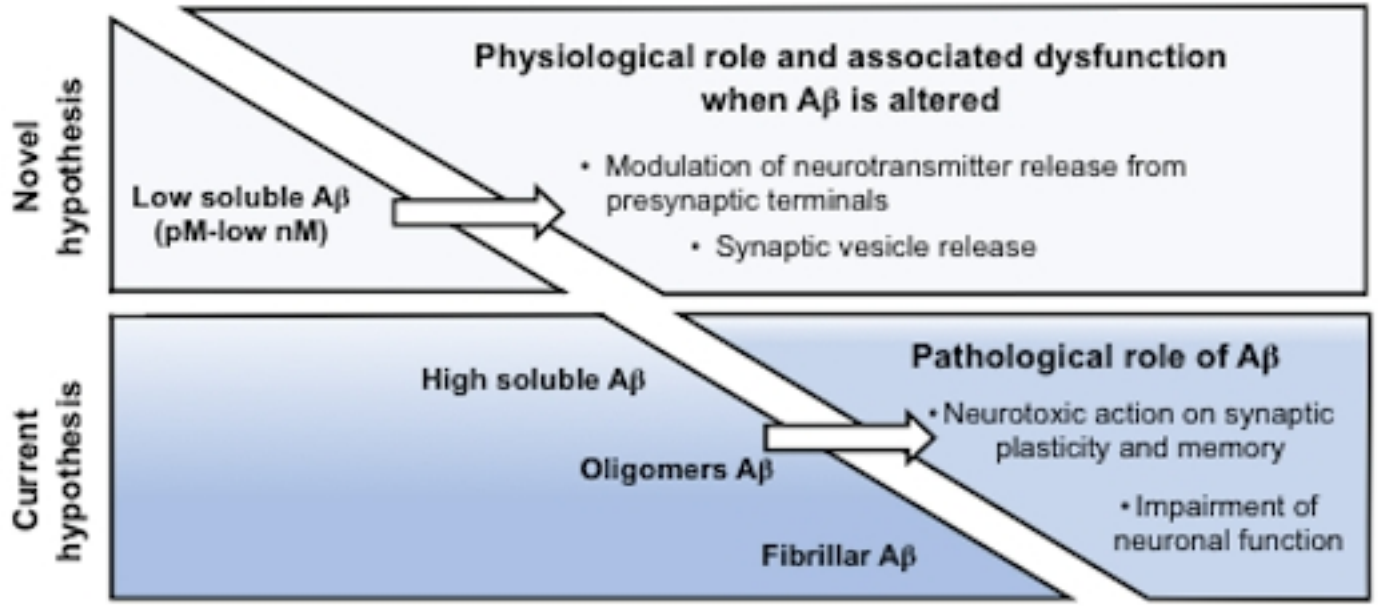
1) The text has been carefully revised for the English language.

2) We made uniform "beta-amyloid" in the paper.

3) We corrected in the abstract section the sentence on beta-amyloid describing it as a peptide.

4) The Reviewer is right and we have provided full word of BACE (β-site APP-cleaving enzyme) in its first appearance in the text (page 6, lane 18).

5) Concerning the crosstalk between serotoninergic transmission and beta-amyloid related to depression and dementia, an explanation is present at page 29, lane 33, page 30, lanes 1-14).


Novel hypothesis
Physiological role and associated dysfunction when Aβ is altered
Low soluble Aβ (pM-low nM)
• Modulation of neurotransmitter release from presynaptic terminals
• Synaptic vesicle release
Current hypothesis
High soluble Aβ
Oligomers Aβ
Fibrillar Aβ
Pathological role of Aβ
• Neurotoxic action on synaptic plasticity and memory
• Impairment of neuronal function

BETA-AMYLOID SHORT- AND LONG-TERM SYNAPTIC ENTANGLEMENT

Cristina Lanni[1], Francesca Fagiani[2], Marco Racchi[1], Stefania Preda[1], Alessia Pascale[1], Massimo Grilli[3], Nicola Allegri[4], Stefano Govoni[1]

[1] Department of Drug Sciences, Pharmacology Section, University of Pavia

[2] Scuola Universitaria Superiore IUSS, Pavia

[3] Pharmacy, Section of Pharmacology and Toxicology, University of Genoa

[4] IRCCS Mondino Foundation, Pavia

Corresponding author:

Stefano Govoni, Ph.D.

Department of Drug Sciences, Pharmacology Section

University of Pavia

Viale Taramelli 14 - 27100, Pavia, Italy

Phone +39 0382 987 394

E-mail govonis@unipv.it

INDEX

ABSTRACT

Beta-amyloid (Aβ) is a peptide that derives from the proteolytic cleavage of the amyloid precursor protein (APP) by several secretases. Since its isolation and sequencing from Alzheimer's disease (AD) brains, Aβ has been intensively investigated in the context of AD as the main pathogenic marker responsible for neurodegenerative processes. During the last three decades, results from several independent studies have converged to form the so-called amyloid cascade hypothesis of AD and several therapeutic strategies designed to modulate the APP amyloidogenic pathway have been developed. However, none of the clinical trials targeting Aβ culminated in a significant clinical outcome, thus challenging the concept that targeting Aβ, at least within the time window so far explored in clinical trials, may have a therapeutic effect. However, besides its presence in AD brains, brain cells produce Aβ, thus suggesting that, under normal conditions, the peptide may have a role in the regulation of brain functions, which is consistent with its ubiquitous presence and normal synthesis. Taking into account that Aβ has been found to exhibit a dual role strictly correlated with its concentration (neuromodulatory/neuroprotective *vs* neurotoxic), we discuss emerging evidence indicating that physiological concentrations of Aβ peptide modulate synaptic activity. The review examines the physiological effects of Aβ on acute synaptic activities and the functional interplay existing between Aβ and different neurotransmitter systems, i.e. cholinergic, glutamatergic, GABAergic, catecholaminergic, serotoninergic, and peptidergic. The review also provides an insight into the different mechanisms through which Aβ affects synaptic activity, focusing in particular on Aβ interaction with the key synaptic proteins that regulate the neurotransmitter release machinery. These interactions may help to identify or recognize alterations in neurotransmitter activity and correlated behaviors as predictive signs for the development of AD and to understand the limitations of current interventions and the failure so far of amyloid targeted therapies.

KEYWORDS: beta-amyloid, acute synaptic activity, neurotransmitter release, SNARE complex, behavioral correlates

1. Introduction

The soluble aggregates of beta-amyloid (Aβ) play a crucial role in the onset of Alzheimer's disease (AD) and have been intensively investigated in the neurodegenerative process within the amyloid cascade hypothesis of AD [1]. Based on this hypothesis, an intense research effort has been directed towards the development of novel therapeutic approaches for the treatment of AD, ranging from strategies specifically targeting the levels of Aβ peptides, either by interfering with their production (e.g. β- and γ-secretase inhibitors) or by enhancing their clearance, to immunotherapy (e.g. humanized antibodies against Aβ peptides). However, all Phase III clinical trials for the treatment of AD failed to meet the desired endpoints, mainly due to a lack of efficacy and/or unexpected side effects.

Despite the failure of these attempts, the validity of the amyloid cascade hypothesis and the role of Aβ peptides in the progression of the disease cannot be discounted. The ineffectiveness of these approaches may depend on two critical factors: the fact that Aβ may not be an ideal druggable target for all AD patients or the wrong timing of therapeutic intervention, a key factor for the success of AD treatment.

The study of specific biomarkers may be useful to better select and stratify patients for an appropriate therapeutic approach. Furthermore, interventions in the early stage of the disease, before the appearance of the first clinical symptoms, may target still reversible pathological alterations.

This time-window based approach makes it necessary to set up new and effective diagnostic tools to detect AD in its prodromal stage. A detailed comprehension of the physiological role of Aβ peptides and their effects on the aging brain might be a starting point to design novel and more efficient therapeutic strategies. Data from literature demonstrate that Aβ peptides exhibit a dual role, i.e. neuromodulatory/neuroprotective *vs* neurotoxic, strictly correlated with their concentration and aggregation state [2,3].

Although the literature defines concentrations of Aβ ranging from picomolar to low nanomolar as physiological, not leading to neurotoxicity, *in vitro* models investigating the effect of synthetic Aβ revealed a great variability in this paradigm. For instance, the issue of extreme supplier-to-supplier and batch-to-batch variability of synthetic peptides is rarely addressed [4]. These limitations, together with the complex dynamic balance existing between Aβ species, contribute to the widespread and controversial literature on Aβ.

The present review examines the effects of exogenous applications of low concentrations of Aβ peptides on the synaptic activity and its functional interplay with different neurotransmitter systems (i.e. cholinergic, glutamatergic, GABAergic, catecholaminergic, serotoninergic and peptidergic systems) (Figure 1). It also explores how Aβ-driven effects may alter neurotransmission over time, possibly contributing to the onset of early neuropsychiatric manifestations such as depression, apathy and psychotic symptoms.

2. Beta-amyloid acute synaptic activities

2.1 Electrophysiology studies

Evidence from the literature shows that Aβ exhibits a dual role that seems to be strictly correlated with its concentration and the age-related cellular environment in the human brain [5]. Low concentrations (picomolar-low nanomolar) of Aβ positively modulate neurotransmission and memory, whereas higher concentrations (high nanomolar-low micromolar) exhibit a neurotoxic and detrimental effect on synaptic plasticity and memory. Furthermore, a regulatory loop has been identified, according to which not only Aβ morphologically and functionally modulates the synapses and synaptic plasticity but also synaptic activity affects Aβ homeostasis [6].

Several *in vitro* [7] and *in vivo* [8] studies have demonstrated that synaptic activity directly regulates the production of Aβ and its release into the extracellular space at the synapses. In the context of amyloid-precursor protein (APP), overexpression in either transgenic (chronic) or virally (acute) driven settings, as well as in the case of endogenous levels of APP, electrophysiological data show that Aβ levels (both $A\beta_{1\text{-}40}$ and the more fibrillogenic $A\beta_{1\text{-}42}$) are significantly modified depending on neuronal electrical activity, whose enhancement promotes Aβ release whereas its reduction has the opposite effect [7,8].

Such results are consistent with the hypothesis that neuronal activity regulates the regional vulnerability to Aβ deposition. Brain areas with high baseline levels of synaptic activity have been found to be more prone to Aβ accumulation [9]. In particular, several studies investigating the impact of neuronal activity on Aβ levels have focused on the default mode network. By using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) with PIB compound, Buckner et al. reported a spatial overlap between the topography of amyloid deposition and the regions of the default mode network (e.g. posterior cingulate and parietal cortex, medial temporal lobe and medial frontal subsystem) [10]. However, not all regions displaying high baseline activity are subject to Aβ

accumulation, so the relationship between neuronal activity and Aβ deposition cannot be considered linear. In a further study by Buckner et al., the strong network connectivity rather than elevated baseline activity has been correlated to regional Aβ deposition. Cortical regions (e.g. posterior cingulate, lateral temporal, lateral parietal, and medial/lateral prefrontal cortices) with intense interconnectivity have been found to display high Aβ deposition [11].

Some of these observations have been supported by parallel *in vitro* and *in vivo* studies, linking APP transport, neuronal activity and Aβ metabolism. APP is axonally transported from the entorhinal cortex to the hippocampal formation through the perforant path [12] and alterations of this pathway result in a decreased Aβ deposition within the hippocampus [13]. The brain regions showing the greatest metabolic activity throughout life – and, most likely, the highest levels of neuronal activity – are the most vulnerable to Aβ accumulation and aggregation in AD patients [10]. Further investigations are needed to better understand if prodromal symptoms of neurodegeneration occur under non-pathological conditions or, alternatively, whether they are involved in the control of altered disease-related behaviors.

It has been hypothesized that the modulation of Aβ secretion by neuronal electrical activity may be mediated by BACE (β-site APP-cleaving enzyme) cleavage at β-secretase sites, though it is still unclear whether neuronal activity influences intrinsic BACE activity or the accessibility of APP to BACE [7]. This hypothesis has not been validated, suggesting that altered BACE-dependent activity is not required for the synaptic activity-dependent Aβ increase [8]. Discrepancies of this kind might reflect differences in experimental settings, implying that different time exposure (short or long term) or areas of infusion might impact on the effect of the neuronal activity on BACE cleavage of APP [14]. Furthermore, considerations on the type of synapses involved in this exploratory mechanism should be made. Differences between low- and high-frequency synapses occur, also depending on the fact that neurotransmitter biosynthesis takes place in the synapses or at more distant sites. A further explanation of the discrepancy of BACE activity in regulating Aβ secretion could be that synaptic activity-dependent Aβ alterations, rather than requiring changes in APP processing, are accomplished via a mechanism specifically related to vesicle fusion. According to this hypothesis, Cirrito et al. defined a pathway by which synaptic activity drives more APP into the endocytic compartment, leading to an enhanced production and release of Aβ [15], thus proving that the increase in Aβ secretion is linked to a higher

presence of APP rather than BACE activity. In particular, it has been suggested that a depolarization of the synaptic terminal might cause calcium influx, leading the synaptic vesicles to fuse with the plasma membrane therefore increasing the amount and rate of endocytosis. Synaptic vesicle membrane recycling via clathrin-mediated endocytosis gives rise to more APP within endosomes, where BACE cleaves APP to release Aβ from the neuron into the brain interstitial fluid [15].

The observation that extracellular Aβ levels are likely to be regulated by synaptic activity suggests that Aβ may be physiologically involved in neuronal processes. Indeed, at physiological levels (picomolar-low nanomolar range), Aβ plays a pivotal role in synaptic structure-functional plasticity, which is crucial to learning and memory. In line with such evidence, healthy murine brains treated with a specific Aβ antibody and an siRNA against murine Aβ showed impaired synaptic plasticity and memory [16]. Subsequent addition of human $A\beta_{1-42}$ rescued these deficits, suggesting that in the healthy brain, physiological Aβ concentrations are necessary for normal synaptic plasticity and memory [16].

Data from studies on APP knock-out (KO) mice with impaired long-term potentiation (LTP) and memory [17] substantiate the involvement of Aβ in hippocampal LTP [2]. In particular, when the Aβ concentration is within the picomolar range, it seems to act as a positive modulator of LTP. The effect of Aβ on LTP has been shown by a dose/response curve, with a postulated biphasic effect of $A\beta_{1-42}$: low concentrations of $A\beta_{1-42}$ induced LTP enhancement at the synapses between Schaeffer collateral fibers and CA1 neurons, with a maximum effect around 200 pM, whereas higher nanomolar $A\beta_{1-42}$ impaired LTP, in line with previous investigations [2,7]. This effect was not obtained either with scrambled $A\beta_{1-42}$, confirming that LTP enhancement is mediated by $A\beta_{1-42}$, or when the peptide was administered after tetanization, supporting the hypothesis that Aβ is required during the induction phase of synaptic plasticity and memory, but not for plasticity maintenance and memory consolidation [16].

The positive action of $A\beta_{1-42}$ on synaptic plasticity has been related to an enhancement of neurotransmitter release during high-frequency stimulation, given that post-tetanic potentiation (a form of short-term plasticity based on the increase of glutamate release from presynaptic terminals due to brief periods of high-frequency stimulation) was increased by perfusion with 200 pM $A\beta_{1-42}$ [2]. However, the N-methyl D-aspartate receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), both implicated in CA-1 LTP, are not involved in Aβ-induced

improvement of synaptic function [18]. Low doses of Aβ did not change current-voltage (I/V) relationships in the NMDA and AMPA receptor current ratio, nor did they alter the amplitude of AMPA receptor-mediated excitatory postsynaptic potentials (EPSCs) or their amplitude distribution [2]. Interestingly, $A\beta_{1-42}$-induced neuroplasticity has been related to α7 nicotinic acetylcholine receptors (α7nAChR), since these effects were absent in α7nAChR knockout mice and blocked by α-Bungarotoxin, a selective antagonist of α7nAChR [2,16].

These data are consistent with the high-binding affinity of Aβ to α7nAChR and the α7nAChR-mediated increase in calcium influx in hippocampal synaptosomes upon infusion of picomolar concentrations of $A\beta_{1-42}$ [19]. The involvement of α7nAChR has recently been shown to be essential in presynaptic function modulation by Aβ: low picomolar $A\beta_{1-40}$ and $A\beta_{1-42}$ increased, whereas endogenous Aβ depletion or application of low micromolar concentrations led to a decrease in the synaptic strength [20], according to data suggesting previous hormetic regulation of neurotransmission by Aβ [2]. These Aβ-induced modulations, in addition to requiring functional α7nAChR, also involved cyclin-dependent kinase 5 (CDK5) and calcineurin signaling, increasing the recycling rate of the synaptic vesicles and supporting the function of Aβ in the regulation of neurotransmitter release [20]. On the other hand, this suggests that a failure of physiological function in synaptic vesicle recycling might be a prodromal marker of cognitive decline and neurodegeneration.

The depression of excitatory synaptic transmission due to high nanomolar concentrations of Aβ, on the other hand, suggested that Aβ may exert a negative feedback function [7]. Following this negative feedback model, intense neuronal activity increases the production of Aβ from endogenous APP and, consequently, Aβ extracellular levels at and near synapses. In turn, Aβ downregulates synaptic transmission, maintaining neuronal activity within a normal dynamic range [7]. This negative feedback process could also operate as a physiological homeostatic mechanism to limit levels of neuronal activity, which, if unchecked, could lead to excitotoxicity. Pathologically aberrant levels of Aβ would be expected to send this negative feedback regulator into overdrive, suppressing excitatory synaptic activity at the postsynaptic level. However, many questions remain unanswered. It is difficult to assign the neurophysiological effects of Aβ to a specific assembly form (soluble monomers and/or soluble oligomers), because these assemblies are likely to exist in a dynamic equilibrium [21]. Aβ conformations released at synapses are still largely

unknown, as is limited our understanding of any age-related change of Aβ species. Aβ conformation following synaptic activity is likely to be a critical factor for the modulation of neurotransmission: nanomolar concentrations of soluble Aβ oligomers, for instance, appear to be much more potent at depressing synaptic transmission than Aβ monomers [22,23]. Given that specific form/s of $A\beta_{1-42}$ (responsible for the enhancing effects on synaptic plasticity) have not been detailed yet, the interpretation of physiological experiments examining synthetic $A\beta_{1-42}$ might be problematic. Moreover, two further hydrophobic residues (i.e. alanine and isoleucine) make $A\beta_{1-42}$ more prone to aggregate than $A\beta_{1-40}$ isoform (even at low concentrations), which is reported to be the most abundant Aβ monomer under physiological conditions in young mammals [21]. Literature on the characterization of synthetic Aβ profile is quite bewildering as it describes different steps of Aβ nucleation between $A\beta_{1-40}$ and $A\beta_{1-42}$ when using synthetic peptides [24].

2.2 Neurochemistry studies

Cholinergic system

Dysfunctional cholinergic transmission is thought to underlie memory impairment and cognitive deficits in AD [25–27]. However, it is still unclear whether this dysfunction is the consequence of the loss of cholinergic neurons and AChRs in the AD brain or a direct effect of molecular interactions of Aβ peptide with AChRs, resulting in a deregulated receptor function. Currently, only few research data explain the putative mechanisms through which physiological Aβ may unbalance the cholinergic system before inducing the loss of cholinergic terminal markers. As several connections between these two key players have been observed, the present review will illustrate the potential interplay linking APP processing, Aβ release and cholinergic receptors before neurodegeneration occurs. Aβ has been reported to interfere with cholinergic neurotransmission by interacting with presynaptic cholinergic receptors function. Notably, both muscarinic and nicotinic receptors are capable of modulating APP processing, diverting its metabolism towards non-amyloidogenic products and promoting the release of the neurotrophic and neuroprotective fragment sAPPα [28].

The activation of specific muscarinic AChRs (mAChRs) M1 and M3 subtypes, mostly distributed in the cerebral cortex and hippocampus, via the stimulation of a downstream signaling pathway involving protein kinase C (PKC), promotes the non-amyloidogenic pathway, concomitantly reducing Aβ production [29]. This evidence is consistent with

several studies demonstrating that direct PKC activation by means of phorbol esters and bryostatin-1 promotes the non-amyloidogenic pathway and decreases Aβ release [30].

The reduction of PKC activity has been associated with all major AD neuropathological markers [31] (although PKC subtype coupled with M1-mAChRs stimulation is still uncertain) and the genetic deletion of M1-mAChRs in $APP_{Swe/Ind}$ mice exacerbates Aβ pathological features [32]. In the regulation of APP metabolism, α4β2- and α7-nAChRs subtypes are also involved. They are known to boost synaptic plasticity and memory [33] and enhance transmitter release in several brain structures including the hippocampus [34,35], spinal cord dorsal horn [36] and amygdala [37]. Nicotinic agonists, nicotine or epibatidine, decrease secretion and intracellular accumulation of Aβ in human SH-EP1 cells stably transfected with both human α4β2-nAChRs and human APP [38].

Furthermore, nicotine increases the release of sAPPα while decreasing Aβ levels in SH-SY5Y cells that express α7-nAChRs, an effect blocked by mecamylamine [39]. Aβ has been reported to induce both activation and inactivation of α7-nAChRs, mostly depending upon the peptide concentration, preparation type (monomers *vs* oligomers) and exposure time [40]. The peptide is also able to interact with α4β2-nAChRs, although its binding affinity for these receptors is 100 to 5000 times lower than α7-nAChRs [41].

Low concentrations (picomolar-low nanomolar) of Aβ activate α7-nAChRs, stimulating signal transduction pathways associated with neuroprotection, synaptic plasticity, learning and memory, mainly in the hippocampal and midbrain dopamine areas [3]. Both $Aβ_{1-40}$ and $Aβ_{1-42}$ isoforms bind to the α7-nAChRs, although $Aβ_{1-40}$ is more effective in competition binding studies compared to $Aβ_{1-40}$. Two mechanisms have been hypothesized in the activation of α7-nAChRs by low concentrations of Aβ: (1) a direct interaction of Aβ with the nicotinic binding site at presynaptic nerve endings of synaptosomes [19] and (2) an indirect modulation of receptor activity as a result of Aβ binding to membrane lipids [42], such as receptor-associated lipid rafts [43].

Higher concentrations (nanomolar-low micromolar) of Aβ or a prolonged exposure to this peptide induce α7-nAChRs desensitization and inactivation, leading to impaired synaptic signaling and neuronal degeneration in response to aversive stimuli. It may be speculated that Aβ physiologically plays a neuromodulatory role on nicotinic receptors, while its accumulation, as occurs in AD, may lead to progressive inactivation of these receptors, thus impairing nicotinic cholinergic transmission. This evidence might suggest that nicotinic agonists are potential agents for AD, although their therapeutic efficacy is limited due to

rapid receptor desensitization. However, compounds that block Aβ binding to nAChRs may limit the sensitization aspects. Recently, Sabec at al. reported that nAChRs in the prefrontal cortex exhibit subtype-specific roles in associative memory encoding and retrieval. In particular, homomeric α7-nAChRs have been demonstrated to be essential for both encoding of associative recognition and induction of LTP, whereas α4β2 subtypes are involved in the retrieval of associative memory and LTD [44]. Given that typical AD patients suffer from memory deficits that specifically affect encoding and storage processes, α7-nAChRs might play a key role in the onset of these deficits.

While there is relatively abundant literature on the direct interactions between Aβ and nicotinic receptors, no reports have been published so far on the direct effects of the peptide on muscarinic recognition sites. Interestingly, Grilli and collaborators have previously demonstrated that Aβ preferentially inhibits the effect of stimulatory mAChRs, leaving the function of inhibitory subtypes unchanged [45]. However, there is no evidence of a direct interaction of Aβ with these receptors and consequently little is known about any Aβ-induced inhibitory mechanisms. *In vitro* studies demonstrated that Aβ at low concentration counteracts muscarinic receptor-activated DA release from dopaminergic terminals by impairing PKC transduction machinery [46]. One might be led to suppose that the effect of Aβ on these mAChRs may be indirect, including the possibility that Aβ may act on an unknown site downstream the muscarinic signal [47].

Besides the effects of the interaction between Aβ and cholinergic receptors, it might also be interesting to investigate the putative effects on direct neuron-to-neuron signaling at the synaptic level. Such an action would be consistent with the localization of AChRs on presynaptic terminals as well as on postsynaptic elements and would argue in favor of short-term functional effects of the peptide. Indeed, while presynaptic nAChRs generally affect (either positively or negatively) neurotransmitter release from presynaptic terminals, M1 and M3 presynaptic receptors stimulate neurotransmitter release both in dopaminergic terminals [46] and GABAergic terminals [45]. Conversely, M2 receptors inhibit neurotransmitter release in cholinergic terminals at the nucleus accumbens [45]. Aβ has been proved to affect the cholinergic control of neurotransmitter release from synaptic terminals, an event that may occur before neurodegeneration.

Aβ-induced modulation/dysfunction in synaptic transmission involves simultaneously different brain transmitters (DA, GABA, glutamate, aspartate, and glycine) and brain areas (nucleus accumbens, striatum, hippocampus), providing grounds for a multi-transmitter

dysregulation hypothesis in the disease. In particular, $A\beta_{1-40}$ concentrations are capable of modulating the release of several neurotransmitters (DA, γ-aminobutyric acid, aspartate, glutamate), elicited by the stimulation of mAChRs and nAChRs subtypes in different brain areas [2,46,48] (see also Table 1).

In the hippocampal region - an early AD target, where the cholinergic pathways are critical for modulation of attention and memory - low Aβ concentrations (100 pM and 1 nM) regulate the nicotine-evoked release of both excitatory (i.e. glutamate and aspartate) and inhibitory aminoacids (i.e. glycine, γ aminobutyric acid) [48,49] (Figure 2). Higher concentrations of $A\beta_{1-40}$ (100 nM and 10 mM administered *in vitro* and *in vivo*, respectively) strongly inhibit the nicotine-elicited release of glutamate and aspartate through the impairment of cholinergic modulation mediated by both α7 and α4β2 receptors [48].

This effect is in line with that shown in the nucleus accumbens and in the striatum in the case of GABA and dopamine release following muscarinic cholinergic stimuli [45,46]. Hence, it can be hypothesized that an early derangement of Aβ production arguably exceeds the threshold beyond which Aβ loses its ability to co-promote the release of aspartate and glutamate (supposedly linked to an efficient memory trace formation) and, subsequently, gains the ability to inhibit the glutamate and aspartate release mediated by cholinergic receptors, thus impairing memory at this point.

Glutamatergic system

Disturbance of excitatory glutamatergic neurotransmission has been linked to several neurodegenerative disorders, including AD [50]. In particular, the exacerbated stimulation of NMDAR is known to mediate excitotoxicity in AD brains, and pyramidal neurons are proposed to be major players in AD-related pathology. Notably, together with acetylcholinesterase inhibitors, the NMDAR noncompetitive antagonist memantine is still an approved choice in the clinical management of AD-type dementia.

Data from the literature strongly support the hypothesis that, in the absence of evident signs of neurotoxicity, Aβ peptides serve a neuromodulatory role on glutamate release, ranging from facilitation to inhibition of stimulated release depending on its concentration.

Recently, Hascup et al. investigated the effect of the local application of human monomeric $A\beta_{1-42}$ on glutamate release in the dentate gyrus, CA3, and CA1 of C57BL/6J mice [51]. Local exposure to different concentrations of $A\beta_{1-42}$ (0.01, 0.1, 1, and 10 μM) has been found to elicit glutamate release in all hippocampal subfields.

Since the concentration of $A\beta_{1-42}$ decreases as the distance from the ejection site increases [52], an approximate concentration of $A\beta_{1-42}$ surrounding MEA (enzyme-based microelectrode array) was calculated. Based on an average distance of 100 microns from the micropipette to the MEA, the concentration of locally-applied $A\beta_{1-42}$ surrounding the MEA has been approximated to be 1, 10, 100, and 1000 nM (for micropipette concentrations of 0.01, 0.1, 1, and 10 μM $A\beta_{1-42}$, respectively) [51].

The application of 100 nM and 1 μM $A\beta_{1-42}$ significantly increased the average maximal amplitude of glutamate release in the dentate gyrus and CA1, while higher concentrations (10 μM) of $A\beta_{1-42}$ were needed in order to increase glutamate release in the dentate gyrus and CA3 [51]. Glutamate release was completely prevented by coapplication of α-Bungarotoxin, thus indicating that monomeric $A\beta_{1-42}$ isoform stimulates glutamate release by acting on α7-nAChRs. Complexively, the above presented data are consistent with the hypothesis that low concentrations (pM-nM) of Aβ positively modulate neurotransmitter release by acting on presynaptic α7-nAChRs.

Accordingly, *in vivo* (microdialysis technique on freely moving rats) and *in vitro* (isolated nerve endings derived from rat hippocampus) experiments support Aβ-driven modulation of glutamate release [48]. Exposure to low concentrations (100 pM and 1 nM) of $A\beta_{1-40}$ peptides has been found to potentiate glutamate and aspartate release elicited by the selective stimulation of α7-nAChRs, thus suggesting a facilitating effect of low concentrations of $A\beta_{1-40}$ on the release of these excitatory aminoacids [48].

In contrast, application of higher concentrations (100 nM and 10 μM, the two highest concentrations respectively used *in vivo* and *in vitro*) of $A\beta_{1-40}$ peptide has been reported to strongly reduce glutamate and aspartate release elicited by nicotine, but not to inhibit the release of glutamate and aspartate evoked by a depolarizing stimulus (veratridine). Such evidence suggests that $A\beta_{1-40}$ at higher concentrations impairs the nicotine-driven neurotransmitter release by directly binding to nAChRs or by indirectly acting downstream on the cellular transduction machinery [48].

The differences between results reported by Hascup et al. and Mura et al. (the inhibition of glutamate release by high concentrations of Aβ peptides observed by these latter) may be due to different methodology and tissue preparation, amyloid delivery techniques and peptide choice. Notably, both groups agree that low concentrations of Aβ peptides might increase glutamate release elicited by α7-nAChRs stimulation.

GABAergic system

In the past, much research focused on the dysfunctions of the glutamatergic and cholinergic neurotransmitter systems in AD, whereas, the inhibitory component of the excitatory/inhibitory network and, particularly, dysfunction in the GABAergic signaling system was poorly investigated. However, GABAergic transmission plays a key role in modulating neuronal responsiveness and excitability [53], network activity [54,55] as well as the maintenance of the excitatory/inhibitory (E/I) balance in the brain [56], which regulates cortical network function. It is well-established that, at early preclinical stages even before amyloid plaque deposition, soluble physiological Aβ peptides impair synaptic transmission by perturbing excitation/inhibition balance [57], inducing neuronal hyperexcitation. A recent work by Ren and coworkers highlighted that Aβ-driven dysfunction of an excitatory/inhibitory balance in key brain areas might represent an early pathological mechanism underlying synaptic impairment and cognitive decline in AD [58]. By using whole-cell recordings in acute mouse brain slices, they demonstrated that the application of low concentrations (50 nM) of $A\beta_{1-42}$ induces hyperexcitability of excitatory pyramidal cells by depressing inhibitory synaptic innervation from fast-spiking interneurons in the anterior cingulate cortex [58], one of the earliest affected areas in AD [59]. Such disruption of GABAergic inhibitory innervation by 50 nM $A\beta_{1-42}$ has been suggested to depend on the perturbation of GABA release from presynaptic terminals. In particular, the excessive activation of dopamine D1 receptors of fast-spiking interneurons has been found to be the main cause contributing to GABAergic input perturbation and, subsequently, to excitation/inhibition imbalance caused by $A\beta_{1-42}$ [58]. Accordingly, the SCH23390 D1 receptor antagonist has been found to reverse $A\beta_{1-42}$-driven perturbation of GABAergic inhibitory input. Therefore, the D1-dependent impairment of fast-spiking GABAergic inhibitory input is likely to serve a key role in $A\beta_{1-42}$-induced excitation/inhibition imbalance in anterior cingulate cortex. Similarly, this excessive dopamine innervation of fast-spiking interneurons in anterior cingulate cortex has been suggested to impair excitation/inhibition balance in schizophrenia [60]. A further contribution to the genesis of psychoses (e.g. schizophrenia and mood disorders with psychotic symptoms) may arise from the impairment of hippocampal GABAergic interneurons and from the subsequent over-activation of neurons that release glutamate into cells located in and projecting from hippocampal CA1 region, in turn impinging upon the dopaminergic control of prefrontal cortex [61]. Therefore, it can be hypothesized that the perturbation of inhibitory synaptic

innervation of pyramidal cells may represent the molecular base underlying the onset of early psychotic symptoms, manifestations of cognitive and perceptual dysfunction (e.g. delusions, hallucinations) also occurring in AD.

Moreover, data from literature have demonstrated that Aβ peptides are capable of affecting GABA release from presynaptic terminals in a concentration-dependent manner. In particular, evidence of the dual effect of Aβ on GABA release derives from *in vivo* studies showing that low concentrations of Aβ peptides have a facilitating action on GABA release, whereas higher concentrations reveal an inhibitor effect [48]. Hippocampal perfusion with 100 nM $A\beta_{1\text{-}40}$ (microdialysis) has been found to elicit a nicotine-evoked GABA overflow while 1 μM $A\beta_{1\text{-}40}$ proved to be ineffective on GABA release and 10 μM $A\beta_{1\text{-}40}$ to inhibit the nicotine-induced release of GABA [48]. This observed dual effect of $A\beta_{1\text{-}40}$ peptides is consistent with the hypothesis that Aβ may serve different biological effects according to the concentration applied, ideally in a continuum from physiology to pathology.

Catecholaminergic system

As regards the catecholaminergic system, several experimental data have explored the involvement of NE and DA in early AD dysfunctions. The present review mainly focuses on recent insights on Aβ interplay with these two neurotransmitters.

Norepinephrine

The locus coeruleus (LC) plays a critical role in modulating arousal, which is important in regulating consciousness, attention, information processing and promoting behaviors such as motor activity, learning and food intake [62]. Despite its well-established role in a plethora of neurodegenerative and neuropsychiatric diseases involving catecholamine neurotransmitters, the LC-NE system has not been thoroughly investigated in relation to AD. From the prodromal stage of the disease, the central noradrenergic system has been demonstrated to undergo substantial changes. Noradrenergic LC cellular and molecular degeneration is a prominent feature of prodromal disease that contributes to cognitive dysfunction, thus supporting a rational basis for targeting LC neuroprotection as a disease-modifying strategy [63]. In human *post-mortem* tissues from subjects who died with a clinical diagnosis of no cognitive impairment (NCI), amnestic mild cognitive impairment (aMCI) or mild/moderate AD, stereologic estimates of total LC neurons revealed a 30%

loss during the transition from NCI to aMCI, with an additional 25% loss of LC neurons in mild/moderate AD [64]. The observed reduction in the number of noradrenergic LC neurons has been significantly associated with worsening *ante-mortem* global cognitive functions as well as poorer performance on neuropsychological tests of episodic memory, semantic memory, working memory, perceptual speed and visuospatial ability [63]. To examine the cellular and molecular pathogenic processes underlying LC neurodegeneration, single population microarray analysis has been performed, revealing significant reductions in select functional classes of mRNAs regulating mitochondrial respiration, redox homeostasis and structural plasticity in neurons from both aMCI and AD subjects compared to NCI. Specific gene expression levels within these functional classes have also been associated with global cognitive deterioration and neuropathological burden [63]. Noradrenergic receptors have been demonstrated to be mainly involved in the regulation of Aβ production. Although extensive research has demonstrated that various G protein-coupled receptors (GPCRs) may influence APP cleavage by promoting or inhibiting α-, β-, γ-secretase activity (see review [65]), it has been shown that β_2 and α_2 adrenergic receptor (ARs) subtypes in the terminal regions of the LC directly affect synaptic transmission and APP processing machinery residing at the synapse, independently of an upsurge in cAMP levels.

The stimulation of β_2ARs has been found to promote Aβ production at noradrenergic synapses. Thathiah et al. suggest that β_2ARs modulate Aβ production via its association with β-arrestin2, which physically interacts with the Aph-1a subunit of the γ-secretase complex, leading to an increase in the catalytic activity of γ-secretase complex [66]. It has been demonstrated, for instance, that β-arrestins are expressed to a greater degree in the brain of AD individuals than in aged-matched controls. Conversely, its production has been found to decrease both in the HEK293-APP_{695} cell line, where *Arrb2* (which encodes β-arrestin2) has been silenced, and in 3-month-old *APP*/*PS1 Arrb2*–/– mice $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ [66]. Ni et al. proposed another potential mechanism through which β_2AR might associate with γ-secretase, namely, via direct binding to PS1 at the plasma membrane [67]. Indeed, following β_2AR stimulation, clathrin-mediated endocytosis of the β_2AR, and the bound to PS1 has been demonstrated. PS1 traffics from the early endosomes to late endosomes and then to lysosomes (LEL), which provide an optimal environment for γ-secretase activity, enhancing its activity and Aβ production [67]. In APPswe/PS1DE9 double-

transgenic mice, chronic treatment with the β_2AR antagonist ICI 118,551 has been demonstrated to reduce amyloid plaque burden [67].

More recently, α_2ARs autoreceptors, coupled with Gi/cAMP systems and regulating NE synthesis and release [68], have been shown to promote amyloidogenic APP processing by blocking the interaction between APP and SorLa, a retromer protein that retains APP in the Golgi compartment under normal physiological conditions [69]. This favours APP transport to endosomal compartments, where it may be proteolytically cleaved. Through the activation of β_2AR on microglia cells, NE has also been found to upregulate the insulin-degrading enzyme (IDE), which, acting also on Aβ, supports the role of NE in modulating Aβ levels at the synapse [70]. Since β_2 and α_2ARs are involved in the modulation of Aβ production and clearance, it might be assumed that an aberrant activation of the LC-NE system during prodromal or early stages of AD before degeneration of LC neurons may contribute to increase Aβ production in LC terminal regions through the stimulation of adrenergic receptors [62]. An open question is whether the aberrant activation of LC and subsequent Aβ increase in projection areas of LC trigger the global dysfunction of LC circuitry.

To further investigate the potential interplay between NE and Aβ, other studies have evaluated the effects of acute soluble Aβ injection on noradrenergic neurotransmission (Table 2). In a rat model, a single i.c.v. injection of $A\beta_{1-42}$ solution (4 μM) has been found to induce a significant increase of NE concentrations in the prefrontal cortex, nucleus accumbens and hippocampus, 2 hours after administration [71]. The mechanism through which Aβ modulates NE concentrations in these areas has not been fully understood. Morgese et al. hypothesized that noradrenergic system activation might be mediated by NO release after NOS induction. The increase in NE concentrations has been associated with higher iNOS mRNA levels and increased NOx concentrations. Furthermore, pharmacological inhibition of the nitrergic system, 30 minutes before Aβ injection, has been found to prevent the increase in NE concentrations [71], suggesting that the effects of Aβ on the noradrenergic system could be associated to NO-related actions.

Dopamine

The dopaminergic system has been involved in the occurrence of cognitive decline, often being predictive of rapidly progressive forms of AD [72]. The integrative properties of the dopaminergic system are probably associated with direct contribution to cognitive

functions at the cortical level, namely in working memory and executive functions. These highly vulnerable functions undergo several changes during the physiological aging process [73] and are severely affected in AD [74]. During aging, in the human caudate putamen, hippocampus and frontal cortex, a reduction has been observed in DA release from its terminals, in D2-subtype receptors and DA transporters, as well as in tyrosine hydroxylase enzyme expression [75]. One of the main correlates of the impairment in DA transmission observed during normal aging is the occurrence of apathy [76], which has been suggested to be a negative prognostic sign in both elderly and AD subjects.

Dopaminergic signals are required for encoding hippocampal memory. In particular, the ventral tegmental area (VTA) and the LC have been described as the primary sources of dopamine acting on dopaminergic receptors in the hippocampus [77]. Nobili et al. investigated alterations of the midbrain dopaminergic system in a Tg2576 mouse model of AD.

They found that an apoptotic process occurred only in the VTA, leading to a progressive loss of the dopaminergic neuronal population, while no Aβ-plaque deposition, hyperphosphorylated tau tangles or any signs of neuronal loss in cortical and hippocampal regions was recorded [78]. In the same model, substantia nigra pars compacta dopaminergic neurons were not affected.

Selective VTA dopaminergic neurons degeneration has been seen to result in lower DA outflow both in the hippocampus and nucleus accumbens shell, brain areas primarily implicated in memory and reward, respectively. Accordingly, the progression of dopaminergic cell death has been correlated with impairments in CA1 synaptic plasticity, memory performance and food reward processing, thus suggesting that degeneration of VTA dopaminergic neurons at pre-plaque stages strongly contributes to memory deficits and dysfunction of reward processing observed in Tg2576 mice [78]. To translate these observations into humans, De Marco and Venneri tested the hypothesis that the volume of the VTA nucleus in humans might be associated with cognitive features of AD, finding that VTA size yields a strong association with hippocampal size and memory performance, particularly in healthy adults [79]. In addition, functional connectivity between the VTA and hippocampus has been reported to be significantly associated with both hippocampal size and memory competence, thus demonstrating that diminished dopaminergic VTA activity may be crucial in the earliest pathological features of AD. Moreover, an interplay between cholinergic and dopaminergic systems seems to play a key role in the modulation of

memory processes and, apparently, their impairment is implicated in the development of AD. A link between these two systems has been demonstrated both in the striatum and in the limbic region showing that ACh promotes the activation of dopaminergic nerve terminals by regulating dopamine release [80]. Accordingly, the cholinergic agonist carbachol has been shown to elicit a robust dopamine release from the shell of nucleus accumbens in freely moving adult rats [46]. The carbachol effect seems to be mainly mediated by the stimulation of cholinergic muscarinic receptors, since the increase of dopamine release is inhibited by the muscarinic antagonist atropine and not by the nicotinic antagonist mecamylamine [46].

In rat nucleus accumbens, 1 μM soluble Aβ infusion through reverse intracerebral dialysis completely counteracted muscarinic receptor-activated DA release in a reversible manner, whereas the overflow of DA elicited by nicotinic receptor activation through epibatidin administration was not altered by Aβ infusion [46]. However, previous results have shown that both nicotinic and muscarinic receptors are equally potent in stimulating dopamine release [81]. In rat nucleus accumbens synaptosomes, the extracellular application of $A\beta_{1-40}$ (100 nM) inhibits both nicotinic and muscarinic cholinergic modulation of DA release by acting from outside and inside the nerve endings respectively [82]. In particular, the inhibition of nicotinic-evoked [^{3}H]DA overflow has been related to the interaction of $A\beta_{1-40}$ with nAChRs through a non-competitive antagonism. On the other hand, the inhibition of muscarinic stimulation of [^{3}H]DA release might be achieved, inside the nerve terminal, through a mechanism which possibly requires the binding of $A\beta_{1-40}$ to a site downstream the mAChR signal. This latter inhibitory effect has been observed at much lower $A\beta_{1-40}$ concentrations (1 nM) than those effective in interfering with nicotinic modulation outside the nerve endings (100 nM) [82].

In line with these findings, a single i.c.v. injection of freshly prepared $A\beta_{1-42}$ (4 μM) has been found to induce a marked reduction in extracellular concentrations of basal DA in the prefrontal cortex when measured 2 hours and 2 days after peptide administration [83] (Table 2). Moreover, the increase in DA release stimulated by local 100 mM K perfusion was abolished in $A\beta_{1-42}$-injected rats [83]. Overall, these results suggest that acute administration of soluble Aβ at concentrations not producing neuronal death inhibits DA release and may serve as a basis for the functional inter-relationship between acute Aβ dysfunction and the vulnerability of dopaminergic transmission in AD.

Serotoninergic system

Serotonergic neurotransmission is critically involved in regulating learning processes and memory storage during adulthood and aging. Pathological changes of 5-HT metabolism and/or an imbalance in serotonergic signaling have been associated with the etiology of various pathophysiological conditions in the CNS, including AD [84]. The possible interplay between serotonergic system and Aβ has been suggested by preclinical experimental data and clinical studies showing that the increase in extracellular serotonin is crucial to modulate Aβ concentrations, by reducing its production from APP or interfering with plaque formation. Administration of the antidepressant citalopram, a selective serotonin reuptake inhibitor (SSRI), has been demonstrated to reduce both $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels in the brain interstitial fluid (ISF) of two- to 3-month-old APP/PS1 transgenic mice [85]. At this age, this mouse model of AD does not yet contain insoluble Aβ deposits. The decrease of Aβ levels occurs almost immediately after drug administration with a significant Aβ reduction starting 12-14 hours after treatment.

As yet, this effect has been evaluated in three different SSRI antidepressants: citalopram (5 mg/kg and 10 mg/kg), fluoxetine (10 mg/kg) and desvenlafaxine (30 mg/kg) [85]. Similarly, direct infusion of serotonin into mouse hippocampus reduced ISF Aβ levels by 35% over an 8-hour period. Moreover, chronic administration of citalopram over a 4-month period has been found to reduce the appearance of new plaques in the 3-month-old PS1APP transgenic mice both in the cortex and hippocampus, compared to control animals [85]. Chronic treatment (5 months) with paroxetine (5 mg/kg), another SSRI, in 5-month-old 3xTg-AD mice reduced AD-related histopathology (Aβ plaques and NFT) in the cortex and the hippocampus and improved memory performance in the Morris spatial navigation task [86]. This suggests that SSRI, administered prophylactically, might retard the disease process and preserve cognitive function.

The beneficial effects of serotonin on Aβ production and concentrations have also been observed in cognitively healthy individuals. In a double-blind study, the acute administration of citalopram in human healthy volunteers, with no prior history of antidepressant treatment, significantly reduced Aβ concentrations in cerebrospinal fluid (CSF) in the citalopram-treated subjects compared to placebo [87]. This suggests a potential preventive approach for AD through reduced Aβ production. Notably, in AD patients the decrease in $A\beta_{1\text{-}42}$ CSF levels may be due, at least in part, to cerebral deposition of Aβ plaques [88]. However, data on the effect at the end of SSRI treatment

are still lacking. Moreover, in a group of patients who had undergone SSRI treatment (mean exposure = 34.5 months) to treat a depressive condition in the five years preceding their enrollment in a positron emission tomography (PET) study with the Pittsburgh Compound B to quantify amyloid binding, lower mean cortical binding potential was observed in comparison with participants who were not exposed to SSRI [85]. Interestingly, the maximal effective dose of citalopram in lowering Aβ brain concentrations and burden in mice (10 mg/kg) is approximately comparable to a dose (50 mg/day) administered to humans as an antidepressant [87]. Yet, a significant difference was found in the timing of the response to SSRI treatment for depression compared to the effect on Aβ concentration.

SSRI treatment of depression generally takes several weeks before amelioration of symptoms occurs, whereas the reduction in CSF Aβ is a short-term effect requiring just a few hours, thus suggesting that the mechanisms by which SSRIs mediate these two effects are different. These observations indicate that the modulation of serotonergic neurotransmission may affect Aβ concentration, probably by decreasing its production without affecting Aβ clearance. This hypothesis has been investigated by Sheline et al. using the incorporation of $^{13}C_6$-Leucine labeled Aβ in healthy subjects treated with citalopram as a tracer of newly-produced Aβ. The tracer/tracee ratio ($^{13}C_6$-Leucine normalized labeled Aβ/unlabeled Aβ) (TTR) over 37 hours of CSF sampling has been found to overlap in the drug-treated and placebo group, thus suggesting that in both groups the fractional turnover of Aβ was comparable [87] and highlighting the effect on Aβ production. To better understand the mechanism underlying this modulation, several studies have evaluated the involvement of serotonin receptor (5-HTRs) subtypes and their signaling pathways. Among the 15 serotonin receptors expressed in the brain, 5-HT2AR, 5-HT2CR, 5-HT4R, 5-HT6R and 5-HT7R have been shown to influence APP processing. Fisher et al. demonstrated that in APP/PS1 mice a specific group of 5-HTRs coupled to G_s proteins (5-HT4R, 5-HT6R and 5-HT7R) is able to suppress Aβ production. Likewise, serotonin or SSRI via microdialysis, 5-HT4R, 5-HT6R and 5-HT7R agonists significantly reduce ISF Aβ [89]. This effect has been supposed to be mediated by the enhancement of APP non-amyloidogenic processing through the increase of α-secretase enzymatic activity [90]. In particular, the activation of 5-HT4R, 5-HT6R and 5-HT7R stimulates G_s proteins, which induce adenylate cyclase (AC) to increase cAMP levels, thus leading to PKA activation. Once activated, PKA through the induction of ERK signaling increases the

ADAM17 cleavage activity by phosphorylation, thus stimulating the release of sAPPα, whose neurotrophic and neuroprotective actions are widely recognized [91]. Interestingly, ADAM10 contains a similar ERK consensus site, thus suggesting that also this member of the ADAM family may be an ERK substrate [92]. In line with these data, mutations on the putative ERK phosphorylation site block the increase of α-secretase enzymatic activity [92], as well as the direct inhibition of MEK by a selective inhibitor (PD98059) which decreases sAPPα release *in vitro* [93].

Besides serotonin, a wide range of extracellular signals can stimulate receptors involved in the activation of ERK-dependent pathways. In particular, the possible involvement of TrkB receptors in modulating ISF Aβ levels has been tested *in vivo* [85]. Treatment with brain-derived neurotrophic factor (BDNF) has not been found to modify ISF Aβ levels in mouse hippocampus [85]. However, several studies have investigated the potential interplay between Aβ, 5-HT and BDNF. Preclinical experimental data have shown that Aβ in its soluble form induces detrimental effects on 5-HT transmission and BDNF content, even before plaque formation and neurodegeneration [94]. Indeed, i.c.v. administration of soluble $A\beta_{1-42}$ peptides (4 μM) has been found to produce functional and biochemical deficits able to induce a depressive-like phenotype in rats [95].

The administration of acute fluoxetine has been demonstrated to restore 5-HT and BDNF levels in soluble Aβ-treated rats, thus significantly improving behavioral performance in forced swimming tests (FST) and reverting depressive soluble Aβ-induced phenotype profiles [95]. This observation is also supported by previous evidence showing a specific fluoxetine-associated neuroprotective effect [96,97]. A prevailing hypothesis suggests that the increase in extracellular 5-HT levels, as would occur upon administration of SSRIs, might increase BDNF levels through 5-HT4, 5-HT6, 5-HT7 receptor subtypes, which are positively coupled to AC and PKA [98]. Also an increase in CREB phosphorylation at ser-133 positively regulates the transcription of BDNF [99]. In particular, by activating the PI3K/AKT pathway, low concentrations of Aβ monomers (100 nM) have been found to induce the activation of CREB and the transcription of the BDNF target gene in differentiated neuroblastoma SH-SY5Y cells and in primary rat cortical neurons [99].

Among the different serotonin receptors, data from literature suggest that targeting 5-HT6R might represent a promising strategy for the symptomatic treatment of AD. In particular, 5-HT6R antagonists have represented a substantial segment of the AD drug development pipeline, with several agents explored with regard to their cognitive enhancing properties

and mechanisms [100]. Even if the activation of 5-HT6R has been found to direct Aβ metabolism towards non-amyloidogenic processing, thus reducing ISF Aβ, the blockade of 5-HT6 receptors has been reported to improve cognition, learning and memory in animal models in a wide variety of learning and memory paradigms [101], with a modest side-effect profile. Such pro-cognitive actions may largely rely on enhancements of cholinergic, glutamatergic, noradrenergic and dopaminergic neurotransmission, at least partly modulated by 5-HT6 receptors [102,103]. Among the developed 5HT6R antagonists, idalopirdine (LU-AE-58054) exhibited a significant benefit on the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) in Phase II [104]. However, in 3 randomized double-blind, placebo-controlled trials, conducted in 2525 patients with mild to moderate AD, the adjunctive use of idalopirdine with cholinesterase inhibitors did not improve cognitive performance or mitigate cognitive decline as measured by the ADAS-Cog total score, over 6 months of treatment [105]. The failure of idalopirdine to meet the expected outcomes suggests a lack of additive efficacy of this combined therapy in the treatment of AD.

Peptidergic system

Neuropeptides are a class of molecules involved in neuron-to-neuron communication. They are found throughout the entire nervous system and act as neurotransmitters, neuromodulators or neurohormones (see review [106]). Inside the nerve cells, neuropeptides are selectively stored within large granular vesicles (LGVs) and commonly coexist in neurons with low-molecular-weight neurotransmitters such as acetylcholine, amino acids and catecholamines. Unlike classical neurotransmitters, neuropeptides have a higher receptor binding affinity and selectivity [106], eliciting their biological effects even when released at lower amounts. The involvement of neuropeptides in brain disorders such as AD was extensively investigated in the nineties, and several studies have currently resumed investigating their role in neurodegeneration (for a review see [107]). This review provides a brief overview about the involvement of some neuropeptides in APP metabolism through their interaction with key enzymes involved in Aβ production and clearance.

Substance P (SP) has been reported to facilitate cognitive functions when directly injected into rat brain regions such as the globus pallidus, central nucleus of amygdala and neostriatum [108] and to play a crucial role not only in memory formation and

reinforcement, but also in preventing memory decline during brain aging. SP is negatively modulated in neurodegenerative disorders such as AD [109], even if no direct evidence for a causative role of SP dysregulation in AD has been demonstrated.

The role of SP as a modulator of Aβ generation has been investigated, and it has been found that SP stimulates APP non-amyloidogenic processing without modifying the steady-state level of APP [110]. Through the binding with NK1 receptors, SP has been demonstrated to reduce Aβ levels by promoting α-secretase-mediated APP cleavage. SP has been seen to specifically increase ADAM9 mRNA and its corresponding protein levels, and to further enhance the amount of the mature form of ADAM10, without modifying its constitutive form [110], thus supporting the previously proposed hypothesis [111] of an upstream activity of ADAM9 on ADAM10 maturation.

A prominent decrease in somatostatin (SST) levels represents another pathological feature of AD. SST has been demonstrated to regulate Aβ metabolism, modulating its proteolytic degradation catalyzed by neprilysin, the major Aβ-degrading enzyme regulating the steady-state levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$. In particular, SST has been shown to significantly increase neprilysin activity in primary murine cortical neuronal cultures, leading to a selective reduction in $A\beta_{1\text{-}42}$ levels in culture media [112]. Moreover, in the hippocampus of SST-knockout mice, neprilysin activity has been found to be altered and a corresponding significant increase in $A\beta_{1\text{-}42}$ levels has been observed [112].

Corticotropin releasing hormone (CRH), another neuropeptide with a central role in stress response through its influence on the hypothalamic-pituitary-adrenal axis, has been found to be reduced in CSF, as well as in the frontal and temporal cortex and caudate nucleus of AD patients. Lezoualc'h et al. demonstrated that CRH promotes the non-amyloidogenic pathway of APP and subsequently increases the secretion of sAPPα in rat cerebellar neurons, in the human neuroblastoma IMR32 cell line and in mouse hippocampal HT22 cells [113]. CRH-stimulated sAPPα-release is blocked by the nonselective CRH receptor antagonist (CRH9–41) and by the selective CRH-R1 antagonist antalarmin, suggesting that the increase in sAPPα release is mediated by the activation of type 1 CRH receptors. However, the specific mechanism through which CRH increases sAPPα secretion has to be further elucidated.

Significant alterations in opioid peptides in AD postmortem brains have been described. CSF β-endorphin levels have been found to be significantly decreased in AD patients compared to controls [114]. In contrast, increased levels of enkephalins - another class of

endogenous opioid peptides modulating functions such as learning, memory, synaptic plasticity and emotional behaviors - have been found in the dentate gyrus of the AD brain compared to controls. Accordingly, in hAPP mice, increased levels of met-enkephalin as well as preproenkephalin mRNA levels have been found in neuronal projections from the entorhinal cortex and dentate gyrus, brain regions involved in memory processes and affected in the early stages of AD [115]. The increase of enkephalin levels, secondary to Aβ infusion, have been correlated with Aβ-induced behavioral alterations and memory deficits observed in hAPP mice [115].

Moreover, increased levels of dynorphin A, but no differences in dynorphin B and nociception, have been found in AD postmortem samples (Broadman area VII) [116]. Opioid receptors have been found to act directly on Aβ production both *in vivo* and *in vitro*. Teng et al. discovered that the δ-opioid receptor (DOR), a GPCR, promotes the amyloidogenic processing of APP. DOR has been shown to form a complex with BACE1 and γ-secretase, promoting APP amyloidogenic processing and Aβ production [117]. The blockage of DOR retards BACE1 and γ-secretase endocytosis and subsequently Aβ production. Consistently, either knockdown or antagonizing DOR have been seen to reduce Aβ production and to ameliorate Aβ pathology by improving cognitive Aβ-dependent deficits in spatial reference memory in APPSWE/PS1 transgenic mice [117].

3. Putative beta-amyloid molecular mechanisms impinging on synaptic activity

Extensive data from the literature demonstrate that synaptic failure precedes cognitive decline in AD [118,119]. However, cellular and molecular events underlying synaptic dysfunction have yet to be fully characterized and understood. This review provides an insight into the different mechanisms through which Aβ affects synaptic activity, focusing on Aβ interaction with key synaptic proteins regulating the neurotransmitter release machinery. Neurotransmitter release is dependent on a tightly coordinated membrane fusion machinery (see review [120]). Exocytosis of synaptic vesicles is mediated by a conserved set of membrane proteins that are commonly known as SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein [SNAP] receptors) [121]. Several studies have shown that, in neurodegenerative diseases such as AD, membrane fusion machinery is strongly altered [122,123] and the formation of the SNARE complex is substantially reduced in the *postmortem* brains of AD patients [124–126]. Furthermore, the deletion of the Munc18-1 gene in mice, codifying for the Munc-18 SNARE protein and

resulting in a genetic ablation of neurotransmitter release, induces pathological similarities to AD, such as altered Tau phosphorylation, neurofibrillary tangles and accumulation of insoluble protein plaques [127].

Aβ has been found to affect SNARE-mediated exocytosis by directly interacting with different synaptic proteins at presynaptic terminals (Table 3). Recently, Yang et al. demonstrated *in vitro* that both Aβ monomers and oligomers are capable of specifically binding to the SNARE motif region (SynH3) of syntaxin 1a [128], which forms a four-helix bundle necessary for membrane fusion [129,130]. However, after binding to the SNARE motif of syntaxin 1a, only the oligomeric form of Aβ (10 μM) has been found to exert an inhibitory effect on SNARE-mediated exocytosis, specifically inhibiting the fusion step between docking and lipid mixing [128]. Otherwise, Aβ monomers failed to exhibit any inhibitory effects on SNARE complex formation or membrane fusion, despite their proven capability to bind to syntaxin 1a.

Another direct interaction between Aβ and synaptic vesicle-associated proteins has been reported by Russel et al. [131] In rat hippocampal neurons, the acute application of low concentrations (50 nM) of $A\beta_{1\text{-}42}$ has been followed by its internalization and localization to presynaptic terminals. In these sites, the peptide interacted with synaptophysin, a synaptic vesicle membrane protein binding synaptobrevin/VAMP2 (vesicle-associated membrane protein) and acting as a control protein thus regulating vesicle fusion [132,133]. $A\beta_{1\text{-}42}$ has been demonstrated to directly compete with VAMP2 for binding synaptophysin at synaptic terminals, thus preventing the formation of synaptophysin/VAMP complex and subsequently inducing the formation of the fusion pore complex followed by neurotransmitter release [131]. Electrophysiology recordings in brain slices confirmed that through this mechanism $A\beta_{1\text{-}42}$ affects baseline transmission. Indeed, in hippocampal slices, the enhancement of single-shock fEPSPs by $A\beta_{1\text{-}42}$ at synapses further suggests an increased availability of releasable synaptic vesicles [131].

In addition to the direct interaction of Aβ with synaptic vesicle proteins regulating neurotransmitter release, an indirect regulation of the release machinery by Aβ might be hypothesized. Data from the literature demonstrate that post-translational modifications of SNARE proteins by protein kinases may influence synaptic vesicle exocytosis. Activation of PKA has been observed to increase exocytosis and neurotransmitter release by phosphorylating synaptic proteins such as SNAP-25, CSPα, synapsin, snapin and RIM1 (Rab interacting molecule) [134,135]. Activation of PKC has also been found to enhance

exocytosis through phosphorylation of SNARE proteins including SNAP-25, Munc-18 and synaptotagmin [136–138]. In particular, a specific phosphorylation site (Ser^{187}) in the SNARE domain of SNAP-25 has been associated with increased exocytosis of synaptic vesicles [139]. Katayama et al. demonstrated that phosphorylation-deficient knock-in (KI) mice, in which SNAP-25 Ser^{187} was replaced with Ala, exhibited an accumulation of synaptic vesicles in enlarged presynaptic terminals and a decreased efficacy of basal synaptic transmission at hippocampal CA1 synapses [140].

Recently, Gao et al. found that phosphorylation of SNAP-25 by PKA and PKC differentially regulates exocytosis of synaptic vesicles and noradrenaline (NA) release in PC12 cells by regulating the SNARE complex assembly [141]. Phosphorylation of SNAP-25 at Ser^{187} by PKC has also been found to enhance Ca^{2+}-dependent release of dopamine and acetylcholine in PC12 cells [142]. On the contrary, phosphorylation of SNAP-25 at Thr^{138} by PKA has been demonstrated to inhibit assembly of the SNARE complex and subsequently NA secretion in PC12 cells [141], although activation of PKA has been widely demonstrated to enhance Ca^{2+}-dependent exocytosis. Taken together these data suggest that phosphorylation of SNARE proteins at specific sites is a key regulatory mechanism through which protein kinases control synaptic vesicle exocytosis and consequently neurotransmitter release.

Given that several data from the literature suggest that Aβ might affect protein kinase transduction machinery, it could be assumed that, by interacting with protein kinases, Aβ might influence phosphorylation of SNARE and accessory proteins as well as the assembly of the SNARE complex, thus modulating neurotransmitter release from presynaptic terminals. This mechanism could explain the previous results, demonstrating that Aβ at low concentrations inhibits the *in vivo* dopamine (DA) release in rat nucleus accumbens and counteracts *in vitro* muscarinic receptor-activated DA release from dopaminergic terminals by impairing PKC transduction machinery [46]. This hypothesis is further supported by *in vitro* results showing that the t-ACPD-induced PKC-mediated release of DA, elicited by presynaptic metabotropic glutamate receptors (mGluRs) located on striatal nerve endings, can be completely antagonized by $Aβ_{1-40}$ [143] (Figure 2). This action has also been demonstrated on signaling cascades downstream mGluRs, where 1 μM Aβ has been reported to impair mGluRs regulation of GABA transmission by inhibiting PKC transduction machinery in prefrontal cortical neurons [144]. Accordingly, Zhong et al. showed that Aβ impairs muscarinic regulation of GABA transmission in prefrontal cortex,

acting on the transduction machinery downstream muscarinic receptors and inhibiting PKC [145].

In addition to PKC and PKA, several other synaptic proteins implicated in synaptic vesicle release and recycling [146,147] are in vitro substrates for various kinases, including the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), the mitogen-activated kinase (MAPK), c-jun N-terminal kinase (JNK) and CDK5. However, their regulatory role in modulating presynaptic transmission and synaptic plasticity has to be fully elucidated. Ninan and Arancio provided direct evidence that presynaptic activation of CaMKII is necessary for inducing synaptic plasticity in cultured hippocampal neurons [148]. In particular, Watanabe et al. suggested that, in the CNS, CaMKII/syntaxin-1A interaction is essential in recruiting complexin, exerting an inhibitory effect on synaptic vesicle fusion, thus inhibiting synaptic vesicle exocytosis and subsequently neurotransmitter release during repetitive stimulation [149].

The phosphorylation state of CaMKII is critical to the functionality of this kinase and decreased levels of active CaMKII at dendritic arborizations may imply impairment of CaMKII synaptic roles. This includes the regulation of synaptic transmission exerted by phosphorylating presynaptic proteins involved in the release machinery. In the hippocampal dentate gyrus, low concentrations of $A\beta_{1-42}$ (200 nM) have been found to inhibit CaMKII activity, through a mechanism involving calcineurin (CaN), serving as a regulator for the phosphorylation state of CaMKII. These data are supported by the observation that CaMKII signaling is dysregulated in aged brain [150] and that, specifically in AD brain, phosphorylated (active) forms of CaMKII significantly decrease in immunoblots of the frontal cortex and hippocampus [151]. In human *post-mortem* brain samples from AD patients, an enhanced expression of phosphorylated/active JNK and a positive co-localization with Aβ have also been identified [152]. The mechanism linking Aβ and JNK has demonstrated that oligomeric $A\beta_{1-42}$ activates JNK, that in turn promotes APP non-amyloidogenic processing, increasing Aβ production [153]. Furthermore, $A\beta_{1-42}$ activates JNK, leading to neurotransmitter release facilitation at presynaptic terminals by affecting the SNARE complex assembly [154]. Overall, the summarized data suggest that Aβ may both impair and stimulate synaptic functions through an action on kinases affecting the SNARE complex activity. The final effect of Aβ depends on its concentration and aggregation state.

4. Tentative behavioral correlates of the Aβ-induced altered neurotransmission

Animal models

The discovery of gene mutations responsible for familial AD made it possible to reproduce some of the specific well-known hallmarks of AD disorder in transgenic animals, including Aβ accumulation. Mice expressing transgenic APP with mutations like Swedish, Indiana, London, Dutch and Flemish, as well as C-terminal fragments of APP have been found to exhibit relevant alterations in several behavioral tasks, similar to behavioral and psychological symptoms (BPSD) observable in AD patients [155]. On the one hand, these observations suggest that changes in behavioral and psychological symptoms of dementia, such as those reported in AD patients, might be partially reproduced in animal models. Other observations highlight that these animal models are not necessarily predictive. To date, there is a lack of in-depth analysis of the alterations in neurotransmission underlying behavioral changes in AD animal models and further investigations are needed to better characterize soluble Aβ-induced behavioral alterations before plaque deposition. Among the different BPSD observed in AD patients, the depressive phenotype is the best characterized in animal models.

Depressive state is considered a prodromal manifestation of the disease before the appearance of cognitive decline symptoms [156], as well as a relevant risk factor for AD [157]. The effect of soluble $A\beta_{1-42}$ peptides has been investigated on working memory, motor activity, anxiety- and depression-related behaviors in young adult male rats on 5-HT neurotransmission and neurotrophin, including BDNF and NGF content in various brain regions. I.c.v. administration of the soluble $A\beta_{1-42}$ peptide appears to induce depressive like-behavior (but not anxiogenic-like phenotype), along with reduced cortical serotonin release and decreased levels of neurotrophines, with no impairment of working memory [157,158]. From a behavioral point of view, soluble Aβ-treated rats exhibited lower exploratory activity, thus suggesting that Aβ might induce motivational deficits before the appearance of cognitive impairments. Moreover, soluble Aβ significantly affected rat behavior during FST by increasing the FST-induced immobility time, thus reflecting a state of behavioral despair or hopelessness [158]. Although obtained in different animal models, these results are consistent with studies reporting that mice overexpressing APP_{SWE}/PS1, at an age characterized by high levels of soluble Aβ, showed an increased duration of immobility in FST [159]. These behavioral alterations induced by soluble $A\beta_{1-42}$ might be

sensitive indicators of early phases of AD and possible risk factors for the development of neuropsychiatric symptoms including depression. Although the mechanism by which soluble Aβ peptide may induce depressive-like behavior has to be fully elucidated, data from the literature suggest that the modulation of 5-HT neurotransmission might be involved [160]. Aβ-induced depressive symptoms might further result in dysfunctions of multiple neurotransmitter systems and in the imbalance of their interactions. Deficits in the dopaminergic system in soluble Aβ-treated rats both in the prefrontal cortex [83] and nucleus accumbens [46] and functional interactions between dopaminergic and 5-HT neuronal systems in the rat prefrontal cortex have been observed. As a possible neuromodulator, soluble Aβ on both the 5-HT and dopaminergic system in the prefrontal cortex might profoundly disrupt the functioning of this area, potentially leading to impairment of mood control. Further investigations are needed to better clarify the molecular mechanism underlying the soluble Aβ-induced depressive phenotype as well as soluble Aβ-induced behavioral alterations mimicking BPSD.

Tentative clinical correlates

In AD, the decline of cognitive functions is accompanied by a complex array of neuropsychiatric symptoms (NPS), also known as “non-cognitive” symptoms of AD. They consist of prominent depression, apathy, agitation, anxiety/phobias, delusions, irritability and sleep disturbances, originally labeled as BPSD (see review [161]). A growing body of evidence emphasizes the importance of NPS as prodromal markers of cognitive decline along the neurodegenerative spectrum. The onset of NPS in MCI patients confers a greater risk of conversion to full-blown dementia compared to MCI patients without NPS [162]. Also, in older adults with normal cognition, the onset of NPS including depression, irritability and agitation has been reported to predict a more rapid cognitive decline compared to subjects without NPS [163], thus suggesting that NPS are prodromal indicators of incipient dementia, measurable even before the onset of MCI. Taragano et al. proposed the expression Mild Behavioral Impairment (MBI) syndrome, not only as a diagnostic construct aimed to identify patients with or without cognitive symptoms, who are prone to develop dementia, but also as a counterpart of MCI, being a transitional state between normal aging and dementia [164].

Brain imaging, electrophysiological, neurochemical and neuropathological approaches have been used as tools to improve the understanding of NPS neurobiology, showing that

atrophy or dysfunction of NPS-relevant brain regions and their related circuits and networks in AD patients are strictly related to the onset of specific cognitive deficits and NPS.

AD affects several brain regions, including the epicenters of emotions and cognition as well as their extensive and reciprocal neuronal connections, thus contributing to the development of both cognitive and NPS-related manifestations [161]. On the other hand, these behavioural symptoms may likely be associated with disease-related synaptic dysfunction rather than neurodegeneration. The subsequent sections will briefly touch upon mounting evidence related to the most prevalent NPS, including apathy, depression, agitation/aggression and psychosis and their related underlying neuropathological and neurotransmitter alterations in AD patients.

Apathy

Among the emotional symptoms observed in MCI and AD patients, apathy has been reported to be the most persistent and common NPS [165]. Data on MCI patients and pre-dementia depressive syndromes suggest that, in prodromal AD, apathy might be linked to dysfunctional affective-emotional processing [166]. This abnormality takes place in the ventromedial prefrontal cortex and in its connections with the amygdala and nucleus accumbens. Consistently, neuropathological progression in AD targets ventromedial parts of the frontal cortex from the early stages of the disease [167]. Evidence from postmortem studies further supports the hypothesis that dopaminergic circuits, linking the basal ganglia with the anterior cingulate and frontal cortices, might be dysfunctional in patients with AD and may account for apathy [72,168].

A reduction in dopamine levels has been observed in the mesolimbic and mesocortical pathways [169], as well as alterations in DA receptor density and localization in apathy-related brain regions in AD patients who experience apathy [170]. In addition, a decrease in blood perfusion to the anterior cingulate [171] and orbitofrontal cortex-areas [172], both innervated by dopaminergic neurons, has been observed. Neuroimaging measures, including magnetic resonance imaging (MRI), single-photon emission CT (SPECT) and F-flurodeoxyglucose (FDG) positron emission tomography (PET), have revealed correlations between apathy and specific neural networks. An MRI study demonstrated a negative correlation between apathy and grey matter volumes in the anterior cingulate and bilateral

frontal cortex [173], whereas FDG-PET investigations reported a correlation between apathy and the left orbitofrontal region [174] and bilateral anterior cingulate region [175].

Depression

Depression is one of the most frequent co-morbid psychiatric disorders in AD, with a prevalence of around 20-50% [176]. The neurobiological and clinical continuum between depression and AD has been suggested by several studies demonstrating that depression might be a relevant risk factor for the development of AD and that the onset of depressive symptoms significantly facilitates the conversion of MCI into AD. Epidemiological evidence and longitudinal studies in MCI and late-life depression (LLD) patients highlighted that depressive disorders represent prodromal manifestations of AD [177]. In addition, studies in earlier-life major depressed (MDD) patients suggested that depression occurring at an early age seems to be an independent risk factor for subsequent AD [178]. However, the neurobiological mechanism underlying this association remains unclear. Depression has been found to share complex pathophysiological routes with dementia. Reduced cortical noradrenergic levels in demented patients with major depression have been observed [179], and a loss of noradrenergic neurons in the LC has been considered an important organic substrate of depression in AD [180]. Furthermore, impaired noradrenergic neurotransmission in the cerebellar cortex might also account for depression in AD [179].
As assessed by PET imaging, a positive correlation between depressive symptoms and cortical amyloid burden has also been observed in the precuneus/posterior cingulate cortex, in cognitively normal subjects with no lifetime history of major depression [181]. This evidence suggests that depressive symptoms might be correlated to Aβ deposition and Aβ-induced synaptic dysfunction during the prodromal phase of the disease.

Agitation and Aggression

Agitation and aggression in people with AD range from 48% to 80% [182]. They have been associated with structural and functional abnormalities in frontal and limbic regions involved in emotional regulation and salience, such as the frontal, anterior cingulate and posterior cingulate cortices, amygdala and hippocampus [183]. Neurochemical studies suggest a link between serotonergic alterations and aggression: reduced levels of 5-HT and its metabolites have been measured in the frontal lobes of aggressive AD patients [184] and an inverse correlation has been found between the levels of the main metabolite

of 5-HT, hippocampal 5-hydroxyindoleacetic acid (5-HIAA) and agitation scores [179]. Dopaminergic alterations might also lie at the basis of aggression/agitation in AD, since an increased cerebellar dopaminergic turnover has been linked to physically-agitated behavior [179]. The observation that dopaminergic turnover correlated with frontal lobe symptoms [179] is potentially indicative of an unbalanced cerebello-thalamic-cortical circuit, since the cerebellum might affect aggressive/agitated behavior in AD by controlling prefrontal circuits [185]. Also, cholinergic modifications are involved in the neurobiology of this specific NPS manifestation, since the treatment with cholinesterase inhibitors significantly improves aggression and agitation in AD patients [167,186].

Psychotic symptoms

Psychosis is common in AD and its major symptoms are delusions, hallucinations and misidentifications. Hallucinations occur less frequently than delusions and are predominantly visual, less commonly auditory and rarely tactile or olfactory [187]. Visual hallucinations in AD patients have been associated with lesions in and atrophy of occipital cortex (visual cortex and association areas), compared to AD patients without visual hallucinations [188]. Delusions have been linked to atrophy of frontal, temporal and limbic regions, including the hippocampus [189]. Derangements in different cerebral circuits have been related to psychosis. A significant reduction in 5-HT levels in the prosubiculum [190], along with a disruption of the noradrenergic locus coeruleus-thalamus system, have been observed. The latter has been argued to potentially lead to psychotic-like behavior, an assumption that has been partially substantiated by the observation that thalamic MHPG (3-methoxy-4-hydroxyphenylglycol, a major noradrenergic metabolite) levels are inversely correlated with hallucinations in AD [179]. Cholinergic alterations have been linked to psychosis, since treatment with cholinesterase inhibitors, besides the well-established benefits on cognition and global function, reduces psychotic symptoms [186]. Finally, decreased dopaminergic neurotransmission and increased dopaminergic catabolism, specifically in the amygdala, have recently been suggested to function as a monoaminergic substrate of psychosis in AD.

Concluding remarks

The reviewed data suggest that Aβ is able to interact with different neurotransmitter release mechanisms in conditions not resulting in neurotoxicity, exerting general effects on

neurotransmission. The modulation of neurotransmitter release from presynaptic terminals by Aβ is mediated by its interaction with specific protein kinases, thus influencing the phosphorylation of SNARE and accessory proteins and subsequently the assembly of the SNARE complex. These effects exerted by physiological concentrations of Aβ over time and their derangement in the disease may disturb neurotransmitter activity, thus contributing to the neuropsychiatric manifestations associated with the disease, such as depression, apathy and psychotic symptoms. In this conceptual frame, the tentative behavioral and clinical correlates strongly suggest a relevant interaction between Aβ metabolism alterations, synaptic activity (including but not limited to synaptic loss) and neuropsychiatric manifestations. These mutual interactions may be useful to identify or recognize alterations in neurotransmitter activity as predictive signs for the development of AD and as a target for pharmacological intervention. Moreover, these observations may explain the limitations of current interventions and the failure so far of amyloid targeted therapies, possibly enabling the preservation of Aβ physiological activity while counteracting its deposition.

FIGURES AND TABLE LEGENDS

Figure 1: APP metabolism. Schematic representation of the non-amyloidogenic and amyloidogenic pathways and definition of the physiological and pathological roles of Aβ fragments generated. The modulation of different neurotransmission systems may affect Aβ production and concentration. The figure shows the mechanisms underlying this modulation, focusing on the involvement of different receptor subtypes and their targets (α-, β or γ-secretase).

Figure 2: Interactions between cholinergic transmission and Aβ. Aβ can interact with both nicotinic and GPCR transmission, exerting different effects depending on its concentration. In particular, low (picomolar to low nanomolar) Aβ concentrations may directly stimulate nicotinic receptors and also facilitate the nicotinic-induced release of excitatory or inhibitory aminoacid transmitters. High Aβ concentrations have been widely demonstrated to inhibit the nicotinic and GPCR-evoked release of several neurotransmitters.

Table 1: Interactions between Aβ and cholinergic receptors in regulating neurotransmitter release.

Table 2: Aβ-induced dysfunctions of different neurotransmitters at synapse level.

Table 3: Direct interplay between Aβ and SNARE proteins.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Cristina Marelli, Ph.D. for her manuscript revision and editorial assistance.

REFERENCES

[1] J. Hardy, The discovery of Alzheimer-causing mutations in the APP gene and the formulation of the “amyloid cascade hypothesis,” FEBS J. (2017). doi:10.1111/febs.14004.

[2] D. Puzzo, L. Privitera, E. Leznik, M. Fa, A. Staniszewski, A. Palmeri, O. Arancio, Picomolar Amyloid- Positively Modulates Synaptic Plasticity and Memory in Hippocampus, J. Neurosci. (2008). doi:10.1523/JNEUROSCI.2692-08.2008.

[3] D. Puzzo, O. Arancio, Amyloid-β Peptide: Dr. Jekyll or Mr. Hyde?, J. Alzheimers. Dis. (2013). doi:10.3233/JAD-2012-129033.

[4] M.Y. Suvorina, O.M. Selivanova, E.I. Grigorashvili, A.D. Nikulin, V. V. Marchenkov, A.K. Surin, O. V. Galzitskaya, Studies of Polymorphism of Amyloid-β42 Peptide from Different Suppliers, J. Alzheimer’s Dis. (2015). doi:10.3233/JAD-150147.

[5] B. Yankner, L. Duffy, D. Kirschner, Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides, Science. (1990). doi:10.1126/science.2218531.

[6] D. Tampellini, Synaptic activity and Alzheimer’s disease: A critical update, Front. Neurosci. (2015). doi:10.3389/fnins.2015.00423.

[7] F. Kamenetz, T. Tomita, H. Hsieh, G. Seabrook, D. Borchelt, T. Iwatsubo, S. Sisodia, R. Malinow, APP Processing and Synaptic Function, Neuron. (2003). doi:10.1016/S0896-6273(03)00124-7.

[8] J.R. Cirrito, K.A. Yamada, M.B. Finn, R.S. Sloviter, K.R. Bales, P.C. May, D.D. Schoepp, S.M. Paul, S. Mennerick, D.M. Holtzman, Synaptic activity regulates interstitial fluid amyloid-β levels in vivo, Neuron. (2005). doi:10.1016/j.neuron.2005.10.028.

[9] A.W. Bero, P. Yan, J.H. Roh, J.R. Cirrito, F.R. Stewart, M.E. Raichle, J.M. Lee, D.M. Holtzman, Neuronal activity regulates the regional vulnerability to amyloid-β? 2 deposition, Nat. Neurosci. (2011). doi:10.1038/nn.2801.

[10] R.L. Buckner, Molecular, Structural, and Functional Characterization of Alzheimer’s Disease: Evidence for a Relationship between Default Activity, Amyloid, and Memory, J. Neurosci. (2005). doi:10.1523/JNEUROSCI.2177-05.2005.

[11] R.L. Buckner, J. Sepulcre, T. Talukdar, F.M. Krienen, H. Liu, T. Hedden, J.R.

Andrews-Hanna, R.A. Sperling, K.A. Johnson, Cortical Hubs Revealed by Intrinsic Functional Connectivity: Mapping, Assessment of Stability, and Relation to Alzheimer's Disease, J. Neurosci. (2009). doi:10.1523/JNEUROSCI.5062-08.2009.

[12] J.D. Buxbaum, G. Thinakaran, V. Koliatsos, J. O'Callahan, H.H. Slunt, D.L. Price, S.S. Sisodia, Alzheimer amyloid protein precursor in the rat hippocampus: transport and processing through the perforant path., J. Neurosci. (1998). doi:10.1042/BST0350416.

[13] O. Lazarov, M. Lee, D. a Peterson, S.S. Sisodia, Evidence that synaptically released beta-amyloid accumulates as extracellular deposits in the hippocampus of transgenic mice. Lazarov, Orly Lee, Michael Peterson, Daniel a Sisodia, Sangram S, J. Neurosci. (2002). doi:22/22/9785 [pii].

[14] B.E. Schroeder, E.H. Koo, To think or not to think: Synaptic activity and Aβ release, Neuron. (2005). doi:10.1016/j.neuron.2005.12.005.

[15] J.R. Cirrito, J.E. Kang, J. Lee, F.R. Stewart, D.K. Verges, L.M. Silverio, G. Bu, S. Mennerick, D.M. Holtzman, Endocytosis Is Required for Synaptic Activity-Dependent Release of Amyloid-β In Vivo, Neuron. (2008). doi:10.1016/j.neuron.2008.02.003.

[16] D. Puzzo, L. Privitera, M. Fa', A. Staniszewski, G. Hashimoto, F. Aziz, M. Sakurai, E.M. Ribe, C.M. Troy, M. Mercken, S.S. Jung, A. Palmeri, O. Arancio, Endogenous amyloid-β is necessary for hippocampal synaptic plasticity and memory, Ann. Neurol. (2011). doi:10.1002/ana.22313.

[17] G.R. Dawson, G.R. Seabrook, H. Zheng, D.W. Smith, S. Graham, G. O'Dowd, B.J. Bowery, S. Boyce, M.E. Trumbauer, H.Y. Chen, L.H.T. Van Der Ploeg, D.J.S. Sirinathsinghji, Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the β-amyloid precursor protein, Neuroscience. (1999). doi:10.1016/S0306-4522(98)00410-2.

[18] J. Lisman, S. Raghavachari, A unified model of the presynaptic and postsynaptic changes during LTP at CA1 synapses., Sci. STKE. (2006). doi:10.1126/stke.3562006re11.

[19] J.J. Dougherty, J. Wu, R.A. Nichols, beta-Amyloid Regulation of Presynaptic Nicotinic Receptors in Rat Hippocampus and Neocortex, J. Neurosci. (2003). doi:10.1073/pnas.1323226111.

[20] F.A. Lazarevic V, Fieńko S, Andres-Alonso M, Anni D, Ivanova D, Montenegro-Venegas C, Gundelfinger ED, Cousin MA, Physiological Concentrations of Amyloid

Beta Regulate Recycling of Synaptic Vesicles via Alpha7 Acetylcholine Receptor and CDK5/Calcineurin Signaling, Front Mol Neurosci. 10:221 (2017).

[21] L. Mucke, D.J. Selkoe, Neurotoxicity of amyloid β-protein: Synaptic and network dysfunction, Cold Spring Harb. Perspect. Med. (2012). doi:10.1101/cshperspect.a006338.

[22] M. Townsend, G.M. Shankar, T. Mehta, D.M. Walsh, D.J. Selkoe, Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: A potent role for trimers, J. Physiol. (2006). doi:10.1113/jphysiol.2005.103754.

[23] C. Holscher, S. Gengler, V.A. Gault, P. Harriott, H.A. Mallot, Soluble beta-amyloid[25-35] reversibly impairs hippocampal synaptic plasticity and spatial learning, Eur. J. Pharmacol. (2007). doi:10.1016/j.ejphar.2007.01.040.

[24] S. Sabella, M. Quaglia, C. Lanni, M. Racchi, S. Govoni, G. Caccialanza, A. Calligaro, V. Belloti, E. De Lorenzi, Capillary electrophoresis studies on the aggregation process of β-amyloid 1-42 and 1-40 peptides, Electrophoresis. (2004). doi:10.1002/elps.200406062.

[25] P. Davies, A.J.F. Maloney, Selective loss of central cholinergic neurons in alzheimer's disease, Lancet. (1976). doi:10.1016/S0140-6736(76)91936-X.

[26] E.K. Perry, B.E. Tomlinson, G. Blessed, K. Bergmann, P.H. Gibson, R.H. Perry, Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia, Br Med J. 2(6150):14 (1978).

[27] P.T. Francis, A.M. Palmer, N.R. Sims, D.M. Bowen, A.N. Davison, M.M. Esiri, D. Neary, J.S. Snowden, G.K. Wilcock, Neurochemical studies of early-onset Alzheimer's disease. Possible influence on treatment, N.Engl.J.Med. (1985). doi:10.1056/NEJM198507043130102.

[28] M. Racchi, M. Sironi, A. Caprera, G. König, S. Govoni, Short- and long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein, Mol. Psychiatry. (2001). doi:10.1038/sj.mp.4000878.

[29] A. Fisher, Cholinergic modulation of amyloid precursor protein processing with emphasis on M1 muscarinic receptor: Perspectives and challenges in treatment of Alzheimer's disease, J. Neurochem. (2012). doi:10.1111/j.1471-4159.2011.07507.x.

[30] M.K. Sun, D.L. Alkon, Bryostatin-1: Pharmacology and therapeutic potential as a CNS drug, CNS Drug Rev. (2006). doi:10.1111/j.1527-3458.2006.00001.x.

[31] D.L. Alkon, M.K. Sun, T.J. Nelson, PKC signaling deficits: a mechanistic hypothesis

for the origins of Alzheimer's disease, Trends Pharmacol. Sci. (2007). doi:10.1016/j.tips.2006.12.002.

[32] A.A. Davis, J.J. Fritz, J. Wess, J.J. Lah, A.I. Levey, Deletion of M1 Muscarinic Acetylcholine Receptors Increases Amyloid Pathology In Vitro and In Vivo, J. Neurosci. (2010). doi:10.1523/JNEUROSCI.6393-09.2010.

[33] D. Ji, R. Lape, J.A. Dani, Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity, Neuron. (2001). doi:10.1016/S0896-6273(01)00332-4.

[34] R. Gray, A.S. Rajan, K.A. Radcliffe, M. Yakehiro, J.A. Dani, Hippocampal synaptic transmission enhanced by low concentrations of nicotine, Nature. (1996). doi:10.1038/383713a0.

[35] K.A. Radcliffe, J.A. Dani, Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission., J. Neurosci. (1998).

[36] J.R. Genzen, D.S. McGehee, Short- and long-term enhancement of excitatory transmission in the spinal cord dorsal horn by nicotinic acetylcholine receptors, Proc Natl Acad Sci U S A. (2003). doi:10.1073/pnas.1131709100\n1131709100 [pii].

[37] R. Girod, N. Barazangi, D. McGehee, L.W. Role, Facilitation of glutamatergic neurotransmission by presynaptic nicotinic acetylcholine receptors, Neuropharmacology. (2000). doi:10.1016/S0028-3908(00)00145-3.

[38] H. Nie, Z. Li, R.J. Lukas, Y. Shen, L. Song, X. Wang, M. Yin, Construction of SH-EP1-α4β2-hAPP695 cell line and effects of nicotinic agonists on β-amyloid in the cells, Cell. Mol. Neurobiol. (2008). doi:10.1007/s10571-007-9218-1.

[39] M. Mousavi, E. Hellström-Lindahl, Nicotinic receptor agonists and antagonists increase sAPPα secretion and decrease Aβ levels in vitro, Neurochem. Int. (2009). doi:10.1016/j.neuint.2008.12.001.

[40] S. Jürgensen, S.T. Ferreira, Nicotinic receptors, amyloid-β, and synaptic failure in Alzheimer's disease, in: J. Mol. Neurosci., 2010. doi:10.1007/s12031-009-9237-0.

[41] H.Y. Wang, D.H.S. Lee, C.B. Davis, R.P. Shank, Amyloid peptide Aβ1-42 binds selectively and with picomolar affinity to α7 nicotinic acetylcholine receptors, J. Neurochem. (2000). doi:10.1046/j.1471-4159.2000.0751155.x.

[42] D.H. Small, D. Maksel, M.L. Kerr, J. Ng, X. Hou, C. Chu, H. Mehrani, S. Unabia, M.F. Azari, R. Loiacono, M.I. Aguilar, M. Chebib, The beta-amyloid protein of Alzheimer's disease binds to membrane lipids but does not bind to the alpha7

nicotinic acetylcholine receptor, J Neurochem. 101:1527–1 (2007).

[43] G.M. Khan, M. Tong, M. Jhun, K. Arora, R.A. Nichols, Beta-Amyloid activates presynaptic alpha7 nicotinic acetylcholine receptors reconstituted into a model nerve cell system: Involvement of lipid rafts, Eur J Neurosci. 31:788–796 (2010).

[44] M.H. Sabec, S. Wonnacott, E.C. Warburton, Z.I. Bashir, Nicotinic Acetylcholine Receptors Control Encoding and Retrieval of Associative Recognition Memory through Plasticity in the Medial Prefrontal Cortex, Cell Rep. (2018). doi:10.1016/j.celrep.2018.03.016.

[45] M. Grilli, F. Lagomarsino, S. Zappettini, S. Preda, E. Mura, S. Govoni, M. Marchi, Specific inhibitory effect of amyloid-β on presynaptic muscarinic receptor subtypes modulating neurotransmitter release in the rat nucleus accumbens, Neuroscience. (2010). doi:10.1016/j.neuroscience.2010.01.058.

[46] S. Preda, S. Govoni, C. Lanni, M. Racchi, E. Mura, M. Grilli, M. Marchi, Acute β-amyloid administration disrupts the cholinergic control of dopamine release in the nucleus accumbens, Neuropsychopharmacology. (2008). doi:10.1038/sj.npp.1301485.

[47] D. V. Janíčková H, Rudajev V, Zimčík P, Jakubík J, Tanila H, El-Fakahany EE, Uncoupling of M1 muscarinic receptor/G-protein interaction by amyloid β(1-42)., Neuropharmacology. 67:272-83. (2013).

[48] E. Mura, S. Zappettini, S. Preda, F. Biundo, C. Lanni, M. Grilli, A. Cavallero, G. Olivero, A. Salamone, S. Govoni, M. Marchi, Dual effect of beta-amyloid on α7 and α4β2 nicotinic receptors controlling the release of glutamate, aspartate and GABA in rat hippocampus, PLoS One. (2012). doi:10.1371/journal.pone.0029661.

[49] S. Zappettini, M. Grilli, G. Olivero, E. Mura, S. Preda, S. Govoni, A. Salamone, M. Marchi, Frontiers: Beta Amyloid Differently Modulate Nicotinic and Muscarinic Receptor Subtypes which Stimulate in vitro and in vivo the Release of Glycine in the Rat Hippocampus, Front. Pharmacol. (2012). doi:10.3389/fphar.2012.00146.

[50] P.T. Francis, N.R. Sims, A.W. Procter, D.M. Bowen, Cortical Pyramidal Neurone Loss May Cause Glutamatergic Hypoactivity and Cognitive Impairment in Alzheimer's Disease: Investigative and Therapeutic Perspectives, J. Neurochem. (1993). doi:10.1111/j.1471-4159.1993.tb13381.x.

[51] K.N. Hascup, E.R. Hascup, Soluble Amyloid-β42 Stimulates Glutamate Release through Activation of the α7 Nicotinic Acetylcholine Receptor, J. Alzheimer's Dis.

(2016). doi:10.3233/JAD-160041.

[52] C. Nicholson, Diffusion and related transport mechanisms in brain tissue, Reports Prog. Phys. (2001). doi:10.1088/0034-4885/64/7/202.

[53] D.A. McCormick, GABA as an inhibitory neurotransmitter in human cerebral cortex, J. Neurophysiol. (1989). doi:10.1152/jn.1989.62.5.1018.

[54] S.R. Cobb, E.H. Buhl, K. Halasy, O. Paulsen, P. Somogyi, Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons, Nature. (1995). doi:10.1038/378075a0.

[55] J. Szabadics, A. Lorincz, G. Tamás, Beta and gamma frequency synchronization by dendritic gabaergic synapses and gap junctions in a network of cortical interneurons., J. Neurosci. (2001). doi:21/15/5824 [pii].

[56] E.O. Mann, M.M. Kohl, O. Paulsen, Distinct Roles of GABAA and GABAB Receptors in Balancing and Terminating Persistent Cortical Activity, J. Neurosci. (2009). doi:10.1523/JNEUROSCI.6162-08.2009.

[57] M.A. Busche, A. Konnerth, Impairments of neural circuit function in Alzheimer's disease, Philos. Trans. R. Soc. B Biol. Sci. (2016). doi:10.1098/rstb.2015.0429.

[58] C.Z. Ren SQ, Yao W, Yan JZ, Jin C, Yin JJ, Yuan J, Yu S, Amyloid β causes excitation/inhibition imbalance through dopamine receptor 1-dependent disruption of fast-spiking GABAergic input in anterior cingulate cortex., Sci Rep. 8(1):302 (2018). doi:doi: 10.1038/s41598-017-18729-5.

[59] H. Braak, E. Braak, Neuropathological stageing of Alzheimer-related changes, Acta Neuropathol. (1991). doi:10.1007/BF00308809.

[60] D.A. Lewis, T. Hashimoto, D.W. Volk, Cortical inhibitory neurons and schizophrenia, Nat. Rev. Neurosci. (2005). doi:10.1038/nrn1648.

[61] J. Lieberman, M. First, Psychotic Disorders, N Engl J Med. 379(3):270 (2018). doi:doi: 10.1056/NEJMra1801490.

[62] J.A. Ross, P. McGonigle, E.J. Van Bockstaele, Locus coeruleus, norepinephrine and Aβ peptides in Alzheimer's disease, Neurobiol. Stress. (2015). doi:10.1016/j.ynstr.2015.09.002.

[63] S.C. Kelly, B. He, S.E. Perez, S.D. Ginsberg, E.J. Mufson, S.E. Counts, Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease, Acta Neuropathol. Commun. (2017). doi:10.1186/s40478-017-0411-2.

[64] R.S. Wilson, S. Nag, P.A. Boyle, L.P. Hizel, L. Yu, A.S. Buchman, J.A. Schneider,

D.A. Bennett, Neural reserve, neuronal density in the locus ceruleus, and cognitive decline, Neurology. (2013). doi:10.1212/WNL.0b013e3182897103.

[65] A. Thathiah, B. De Strooper, The role of G protein-coupled receptors in the pathology of Alzheimer's disease, Nat. Rev. Neurosci. (2011). doi:10.1038/nrn2977.

[66] A. Thathiah, K. Horré, A. Snellinx, E. Vandewyer, Y. Huang, M. Ciesielska, G. De Kloe, S. Munck, B. De Strooper, β-Arrestin 2 regulates Aβ generation and γ-secretase activity in Alzheimer's disease, Nat. Med. (2013). doi:10.1038/nm.3023.

[67] Y. Ni, X. Zhao, G. Bao, L. Zou, L. Teng, Z. Wang, M. Song, J. Xiong, Y. Bai, G. Pei, Activation of β2-adrenergic receptor stimulates γ-secretase activity and accelerates amyloid plaque formation, Nat. Med. (2006). doi:10.1038/nm1485.

[68] C.W. Berridge, B.D. Waterhouse, The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes, Brain Res. Rev. (2003). doi:10.1016/S0165-0173(03)00143-7.

[69] Y. Chen, Y. Peng, P. Che, M. Gannon, Y. Liu, L. Li, G. Bu, T. van Groen, K. Jiao, Q. Wang, α_{2A} adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction, Proc. Natl. Acad. Sci. (2014). doi:10.1073/pnas.1409513111.

[70] Y. Kong, L. Ruan, L. Qian, X. Liu, Y. Le, Norepinephrine Promotes Microglia to Uptake and Degrade Amyloid Peptide through Upregulation of Mouse Formyl Peptide Receptor 2 and Induction of Insulin-Degrading Enzyme, J. Neurosci. (2010). doi:10.1523/JNEUROSCI.2985-10.2010.

[71] M.G. Morgese, M. Colaianna, E. Mhillaj, M. Zotti, S. Schiavone, P. D'Antonio, A. Harkin, V. Gigliucci, P. Campolongo, V. Trezza, A. De Stradis, P. Tucci, V. Cuomo, L. Trabace, Soluble beta amyloid evokes alteration in brain norepinephrine levels: Role of nitric oxide and interleukin-1, Front. Neurosci. (2015). doi:10.3389/fnins.2015.00428.

[72] A. Martorana, G. Koch, Is dopamine involved in Alzheimer's disease?, Front. Aging Neurosci. (2014). doi:10.3389/fnagi.2014.00252.

[73] A.F.T. Arnsten, B.M. Li, Neurobiology of executive functions: Catecholamine influences on prefrontal cortical functions, Biol. Psychiatry. (2005). doi:10.1016/j.biopsych.2004.08.019.

[74] S. Baudic, G.D. Barba, M.C. Thibaudet, A. Smagghe, P. Remy, L. Traykov, Executive function deficits in early Alzheimer's disease and their relations with episodic memory, Arch. Clin. Neuropsychol. (2006). doi:10.1016/j.acn.2005.07.002.

[75] L. Bäckman, U. Lindenberger, S.C. Li, L. Nyberg, Linking cognitive aging to alterations in dopamine neurotransmitter functioning: Recent data and future avenues, Neurosci. Biobehav. Rev. (2010). doi:10.1016/j.neubiorev.2009.12.008.

[76] P.H. Robert, E. Mulin, P. Malléa, R. David, Apathy diagnosis, assessment, and treatment in Alzheimer's disease, CNS Neurosci. Ther. (2010). doi:10.1111/j.1755-5949.2009.00132.x.

[77] M. D'Amelio, L. Serra, M. Bozzali, Ventral Tegmental Area in Prodromal Alzheimer's Disease: Bridging the Gap between Mice and Humans, J. Alzheimer's Dis. (2018). doi:10.3233/JAD-180094.

[78] A. Nobili, E.C. Latagliata, M.T. Viscomi, V. Cavallucci, D. Cutuli, G. Giacovazzo, P. Krashia, F.R. Rizzo, R. Marino, M. Federici, P. De Bartolo, D. Aversa, M.C. Dell'Acqua, A. Cordella, M. Sancandi, F. Keller, L. Petrosini, S. Puglisi-Allegra, N.B. Mercuri, R. Coccurello, N. Berretta, M. D'Amelio, Dopamine neuronal loss contributes to memory and reward dysfunction in a model of Alzheimer's disease, Nat. Commun. (2017). doi:10.1038/ncomms14727.

[79] M. De Marco, A. Venneri, Volume and connectivity of the ventral tegmental area are linked to neurocognitive signatures of Alzheimer's disease in humans, J. Alzheimer's Dis. (2018). doi:10.3233/JAD-171018.

[80] W. Zhang, M. Yamada, J. Gomeza, A.S. Basile, J. Wess, Multiple Muscarinic Acetylcholine Receptor Subtypes Modulate Striatal Dopamine Release, as Studied with M1-M5 Muscarinic Receptor Knock-Out Mice, J. Neurosci. (2002). doi:20026644.

[81] M. Grilli, M. Parodi, M. Raiteri, M. Marchi, Chronic nicotine differentially affects the function of nicotinic receptor subtypes regulating neurotransmitter release, J. Neurochem. (2005). doi:10.1111/j.1471-4159.2005.03126.x.

[82] G. Olivero, M. Grilli, J. Chen, S. Preda, E. Mura, S. Govoni, M. Marchi, Effects of soluble β-amyloid on the release of neurotransmitters from rat brain synaptosomes, Front. Aging Neurosci. (2014). doi:10.3389/fnagi.2014.00166.

[83] L. Trabace, K.M. Kendrick, S. Castrignanò, M. Colaianna, A. De Giorgi, S. Schiavone, C. Lanni, V. Cuomo, S. Govoni, Soluble amyloid beta1-42 reduces dopamine levels in rat prefrontal cortex: Relationship to nitric oxide, Neuroscience. (2007). doi:10.1016/j.neuroscience.2007.04.056.

[84] M. Butzlaff, E. Ponimaskin, The role of Serotonin Receptors in Alzheimer's disease,

Opera Med Physiol. (2016). doi:10.1016/S0214-4603(91)75503-4.

[85] J.R. Cirrito, B.M. Disabato, J.L. Restivo, D.K. Verges, W.D. Goebel, A. Sathyan, D. Hayreh, G. D'Angelo, T. Benzinger, H. Yoon, J. Kim, J.C. Morris, M.A. Mintun, Y.I. Sheline, Serotonin signaling is associated with lower amyloid-β levels and plaques in transgenic mice and humans, Proc. Natl. Acad. Sci. (2011). doi:10.1073/pnas.1107411108.

[86] R.L. Nelson, Z. Guo, V.M. Halagappa, M. Pearson, A.J. Gray, Y. Matsuoka, M. Brown, B. Martin, T. Iyun, S. Maudsley, R.F. Clark, M.P. Mattson, Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice, Exp. Neurol. (2007). doi:10.1016/j.expneurol.2007.01.037.

[87] Y.I. Sheline, T. West, K. Yarasheski, R. Swarm, M.S. Jasielec, J.R. Fisher, W.D. Ficker, P. Yan, C. Xiong, C. Frederiksen, M. V. Grzelak, R. Chott, R.J. Bateman, J.C. Morris, M.A. Mintun, J.M. Lee, J.R. Cirrito, An antidepressant decreases CSF Aβ production in healthy individuals and in transgenic AD mice., Sci. Transl. Med. 6, 236re23 (2014).

[88] B. Dubois, H.H. Feldman, C. Jacova, S.T. DeKosky, P. Barberger-Gateau, J. Cummings, A. Delacourte, D. Galasko, S. Gauthier, G. Jicha, K. Meguro, J. O'Brien, F. Pasquier, P. Robert, M. Rossor, S. Salloway, Y. Stern, P.J. Visser, P. Scheltens, Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria, Lancet Neurol. (2007). doi:10.1016/S1474-4422(07)70178-3.

[89] J.R. Fisher, C.E. Wallace, D.L. Tripoli, Y.I. Sheline, J.R. Cirrito, Redundant Gs-coupled serotonin receptors regulate amyloid-β metabolism in vivo, Mol. Neurodegener. (2016). doi:10.1186/s13024-016-0112-5.

[90] G. Hashimoto, M. Sakurai, A.F. Teich, F. Saeed, F. Aziz, O. Arancio, 5-HT4receptor stimulation leads to soluble aβPPα production through MMP-9 upregulation, J. Alzheimer's Dis. (2012). doi:10.3233/JAD-2012-111235.

[91] M. Cochet, R. Donneger, E. Cassier, F. Gaven, S.F. Lichtenthaler, P. Marin, J. Bockaert, A. Dumuis, S. Claeysen, 5-HT4receptors constitutively promote the non-amyloidogenic pathway of APP cleavage and interact with ADAM10, ACS Chem. Neurosci. (2013). doi:10.1021/cn300095t.

[92] M. Cisse, U. Braun, M. Leitges, A. Fisher, G. Pages, F. Checler, B. Vincent, ERK1-independent α-secretase cut of β-amyloid precursor protein by M1 muscarinic

receptors and PKCs., Mol. Cell. Neurosci. (2011). doi:10.1016/j.mcn.2011.04.008.

[93] L.F. Norum JH, Hart K, Ras-dependent ERK activation by the human G(s)-coupled serotonin receptors 5-HT4(b) and 5-HT7(a), J Biol Chem. 278:3098–1 (2003).

[94] D. Moechars, K. Lorent, F. Van Leuven, Premature death in transgenic mice that overexpress a mutant amyloid precursor protein is preceded by severe neurodegeneration and apoptosis, Neuroscience. (1999). doi:10.1016/S0306-4522(98)00599-5.

[95] S. Schiavone, P. Tucci, E. Mhillaj, M. Bove, L. Trabace, M.G. Morgese, Antidepressant drugs for beta amyloid-induced depression: A new standpoint?, Prog. Neuro-Psychopharmacology Biol. Psychiatry. (2017). doi:10.1016/j.pnpbp.2017.05.004.

[96] F. Caraci, F. Tascedda, S. Merlo, C. Benatti, S.F. Spampinato, A. Munafò, G.M. Leggio, F. Nicoletti, N. Brunello, F. Drago, M.A. Sortino, A. Copani, Fluoxetine prevents Aβ1-42-induced toxicity via a paracrine signaling mediated by transforming-growth-factor-β1, Front. Pharmacol. (2016). doi:10.3389/fphar.2016.00389.

[97] L. Jin, L.F. Gao, D.S. Sun, H. Wu, Q. Wang, D. Ke, H. Lei, J.Z. Wang, G.P. Liu, Long-term Ameliorative Effects of the Antidepressant Fluoxetine Exposure on Cognitive Deficits in 3 × TgAD Mice, Mol. Neurobiol. (2017). doi:10.1007/s12035-016-9952-9.

[98] K. Martinowich, B. Lu, Interaction between BDNF and serotonin: Role in mood disorders, Neuropsychopharmacology. (2008). doi:10.1038/sj.npp.1301571.

[99] S. Zimbone, I. Monaco, F. Gianì, G. Pandini, A.G. Copani, M.L. Giuffrida, E. Rizzarelli, Amyloid Beta monomers regulate cyclic adenosine monophosphate response element binding protein functions by activating type-1 insulin-like growth factor receptors in neuronal cells, Aging Cell. (2018). doi:10.1111/acel.12684.

[100] J. Cummings, G. Lee, A. Ritter, K. Zhong, Alzheimer's disease drug development pipeline: 2018, Alzheimer's Dement. Transl. Res. Clin. Interv. (2018). doi:10.1016/j.trci.2018.03.009.

[101] J. Arnt, B. Bang-Andersen, B. Grayson, F.P. Bymaster, M.P. Cohen, N.W. Delapp, B. Giethlen, M. Kreilgaard, D.L. McKinzie, J.C. Neill, D.L. Nelson, S.M. Nielsen, M.N. Poulsen, J.M. Schaus, L.M. Witten, Lu AE58054, a 5-HT6 antagonist, reverses cognitive impairment induced by subchronic phencyclidine in a novel object

recognition test in rats, Int. J. Neuropsychopharmacol. (2010). doi:10.1017/S1461145710000659.

[102] L.A. Dawson, H.Q. Nguyen, P. Li, The 5-HT6receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus, Neuropsychopharmacology. (2001). doi:10.1016/S0893-133X(01)00265-2.

[103] A. Mørk, R.V. Russell, I.E.M. de Jong, G. Smagin, Effects of the 5-HT6receptor antagonist idalopirdine on extracellular levels of monoamines, glutamate and acetylcholine in the rat medial prefrontal cortex, Eur. J. Pharmacol. (2017). doi:10.1016/j.ejphar.2017.02.010.

[104] Wilkinson D, Windfeld K, E. Colding-Jørgensen, Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial, Lancet Neurol., (2014), 13(11):109.

[105] A. Atri, L. Frölich, C. Ballard, P.N. Tariot, J.L. Molinuevo, N. Boneva, K. Windfeld, L.L. Raket, J.L. Cummings, Effect of idalopirdine as adjunct to cholinesterase inhibitors on change in cognition in patients with Alzheimer disease three randomized clinical trials, in: JAMA - J. Am. Med. Assoc., (2018). doi:10.1001/jama.2017.20373.

[106] A. Merighi, C. Salio, F. Ferrini, L. Lossi, Neuromodulatory function of neuropeptides in the normal CNS, J. Chem. Neuroanat. (2011). doi:10.1016/j.jchemneu.2011.02.001.

[107] D. Van Dam, A. Van Dijck, L. Janssen, P.P. De Deyn, Neuropeptides in Alzheimer`s Disease: From Pathophysiological Mechanisms to Therapeutic Opportunities, Curr. Alzheimer Res. (2013). doi:10.2174/1567205011310050001.

[108] L. Liu, M. Shenoy, P.J. Pasricha, Substance P and calcitonin gene related peptide mediate pain in chronic pancreatitis and their expression is driven by nerve growth factor, J. Pancreas. (2011).

[109] C. Severini, C. Petrella, P. Calissano, Substance P and Alzheimer's Disease: Emerging Novel Roles, Curr. Alzheimer Res. (2016). doi:10.2174/1567205013666160401114039.

[110] R. Marolda, M.T. Ciotti, C. Matrone, R. Possenti, P. Calissano, S. Cavallaro, C. Severini, Substance P activates ADAM9 mRNA expression and induces α-

secretase-mediated amyloid precursor protein cleavage, Neuropharmacology. (2012). doi:10.1016/j.neuropharm.2011.12.025.

[111] T. Tousseyn, A. Thathiah, E. Jorissen, T. Raemaekers, U. Konietzko, K. Reiss, E. Maes, A. Snellinx, L. Serneels, O. Nyabi, W. Annaert, P. Saftig, D. Hartmann, B. de Strooper, ADAM10, the rate-limiting protease of regulated intramembrane proteolysis of notch and other proteins, is processed by ADAMS-9, ADAMS-15, and the γ-secretase, J. Biol. Chem. (2009). doi:10.1074/jbc.M805894200.

[112] T. Saito, N. Iwata, S. Tsubuki, Y. Takaki, J. Takano, S.M. Huang, T. Suemoto, M. Higuchi, T.C. Saido, Somatostatin regulates brain amyloid β peptide Aβ42 through modulation of proteolytic degradation, Nat. Med. (2005). doi:10.1038/nm1206.

[113] F. Lezoualc'h, Corticotropin-Releasing Hormone-Mediated Neuroprotection against Oxidative Stress Is Associated with the Increased Release of Non-amyloidogenic Amyloid Precursor Protein and with the Suppression of Nuclear Factor- B, Mol. Endocrinol. (2000). doi:10.1210/me.14.1.147.

[114] L.T. Sulkava R, Erkinjuntti T, CSF beta-endorphin and beta-lipotropin in Alzheimer's disease and multi-infarct dementia, Neurology. 35(7) (1985) 1057–8.

[115] W.J. Meilandt, G.-Q. Yu, J. Chin, E.D. Roberson, J.J. Palop, T. Wu, K. Scearce-Levie, L. Mucke, Enkephalin Elevations Contribute to Neuronal and Behavioral Impairments in a Transgenic Mouse Model of Alzheimer's Disease, J. Neurosci. (2008). doi:10.1523/JNEUROSCI.0590-08.2008.

[116] T. Yakovleva, Z. Marinova, A. Kuzmin, N.G. Seidah, V. Haroutunian, L. Terenius, G. Bakalkin, Dysregulation of dynorphins in Alzheimer disease, Neurobiol. Aging. (2007). doi:10.1016/j.neurobiolaging.2006.07.002.

[117] P.G. Teng L, Zhao J, Wang F, Ma L, A GPCR/secretase complex regulates beta- and gamma-secretase specificity for Abeta production and contributes to AD pathogenesis, Cell Res. 20(2):138- (2010).

[118] V. Nimmrich, U. Ebert, Is alzheimer's disease a result of presynaptic failure? - Synaptic dysfunctions induced by oligomeric p-amyloid, Rev. Neurosci. (2009). doi:10.1515/REVNEURO.2009.20.1.1.

[119] D.J. Selkoe, Alzheimer's disease is a synaptic failure, Science (80-.). (2002). doi:10.1126/science.1074069.

[120] T.C. Südhof, J. Rizo, Synaptic vesicle exocytosis, Cold Spring Harb. Perspect. Biol. (2011). doi:10.1101/cshperspect.a005637.

[121] T.C. Südhof, J.E. Rothman, Membrane fusion: Grappling with SNARE and SM proteins, Science. (2009). doi:10.1126/science.1161748.

[122] P. Garcia-Reitböck, O. Anichtchik, A. Bellucci, M. Iovino, C. Ballini, E. Fineberg, B. Ghetti, L. Della Corte, P. Spano, G.K. Tofaris, M. Goedert, M.G. Spillantini, SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease, Brain. (2010). doi:10.1093/brain/awq132.

[123] J. Shen, S.S. Rathore, L. Khandan, J.E. Rothman, SNARE bundle and syntaxin N-peptide constitute a minimal complement for Munc18-1 activation of membrane fusion, J. Cell Biol. (2010). doi:10.1083/jcb.201003148.

[124] M. Sharma, J. Burré, P. Bronk, Y. Zhang, W. Xu, T.C. Südhof, CSPα knockout causes neurodegeneration by impairing SNAP-25 function, EMBO J. (2012). doi:10.1038/emboj.2011.467.

[125] W.G. Honer, A.M. Barr, K. Sawada, A.E. Thornton, M.C. Morris, S.E. Leurgans, J.A. Schneider, D.A. Bennett, Cognitive reserve, presynaptic proteins and dementia in the elderly, Transl. Psychiatry. (2012). doi:10.1038/tp.2012.38.

[126] E.B. Mukaetova-Ladinska, F. Garcia-Siera, J. Hurt, H.J. Gertz, J.H. Xuereb, R. Hills, C. Brayne, F.A. Huppert, E.S. Paykel, M. McGee, R. Jakes, W.G. Honer, C.R. Harrington, C.M. Wischik, Staging of cytoskeletal and β-amyloid changes in human isocortex reveals biphasic synaptic protein response during progression of Alzheimer's disease, Am. J. Pathol. (2000). doi:10.1016/S0002-9440(10)64573-7.

[127] C. Law, M. Schaan Profes, M. Levesque, J.A. Kaltschmidt, M. Verhage, A. Kania, Normal Molecular Specification and Neurodegenerative Disease-Like Death of Spinal Neurons Lacking the SNARE-Associated Synaptic Protein Munc18-1, J Neurosci. 13;36(2):5 (2016). doi: 10.1523/JNEUROSCI.1964-15.2016.

[128] Y. Yang, J. Kim, H.Y. Kim, N. Ryoo, S. Lee, Y.S. Kim, H. Rhim, Y.K. Shin, Amyloid-β Oligomers May Impair SNARE-Mediated Exocytosis by Direct Binding to Syntaxin 1a, Cell Rep. (2015). doi:10.1016/j.celrep.2015.07.044.

[129] M.A. Poirier, W. Xiao, J.C. Macosko, C. Chan, Y.K. Shin, M.K. Bennett, The synaptic SNARE complex is a parallel four-stranded helical bundle, Nat. Struct. Biol. (1998). doi:10.1038/1799.

[130] R.B. Sutton, D. Fasshauer, R. Jahn, A.T. Brunger, Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution, Nature. (1998). doi:10.1038/26412.

[131] C.L. Russell, S. Semerdjieva, R.M. Empson, B.M. Austen, P.W. Beesley, P. Alifragis, Amyloid-β acts as a regulator of neurotransmitter release disrupting the interaction between synaptophysin and VAMP2, PLoS One. (2012). doi:10.1371/journal.pone.0043201.

[132] R.E. Leube, P. Kaiser, A. Seiter, R. Zimbelmann, W.W. Franke, H. Rehm, P. Knaus, P. Prior, H. Betz, H. Reinke, Synaptophysin: molecular organization and mRNA expression as determined from cloned cDNA, EMBO J. (1987).

[133] B. Wiedenmann, W.W. Franke, Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles, Cell. (1985). doi:10.1016/S0092-8674(85)80082-9.

[134] G. Lonart, S. Schoch, P.S. Kaeser, C.J. Larkin, T.C. Südhof, D.J. Linden, Phosphorylation of RIM1α by PKA triggers presynaptic long-term potentiation at cerebellar parallel fiber synapses, Cell. (2003). doi:10.1016/S0092-8674(03)00727-X.

[135] J. Boczan, A.G.M. Leenders, Z.H. Sheng, Phosphorylation of Syntaphilin by cAMP-dependent Protein Kinase Modulates Its Interaction with Syntaxin-1 and Annuls Its Inhibitory Effect on Vesicle Exocytosis, J. Biol. Chem. (2004). doi:10.1074/jbc.M400496200.

[136] G. Nagy, K. Reim, U. Matti, N. Brose, T. Binz, J. Rettig, E. Neher, J.B. Sørensen, Regulation of Releasable Vesicle Pool Sizes by Protein Kinase A-Dependent Phosphorylation of SNAP-25, Neuron. (2004). doi:10.1016/S0896-6273(04)00038-8.

[137] R. Hepp, J.P. Cabaniols, P.A. Roche, Differential phosphorylation of SNAP-25 in vivo by protein kinase C and protein kinase A, FEBS Lett. (2002). doi:10.1016/S0014-5793(02)03629-3.

[138] J. Fu, A.P. Naren, X. Gao, G.U. Ahmmed, A.B. Malik, Protease-activated receptor-1 activation of endothelial cells induces protein kinase Cα-dependent phosphorylation of syntaxin 4 and Munc18c: Role in signaling P-selectin expression, J. Biol. Chem. (2005). doi:10.1074/jbc.M410044200.

[139] Y. Shu, X. Liu, Y. Yang, M. Takahashi, K.D. Gillis, Phosphorylation of SNAP-25 at Ser187 Mediates Enhancement of Exocytosis by a Phorbol Ester in INS-1 Cells, J. Neurosci. (2008). doi:10.1523/JNEUROSCI.2352-07.2008.

[140] N. Katayama, S. Yamamori, M. Fukaya, S. Kobayashi, M. Watanabe, M. Takahashi, T. Manabe, SNAP-25 phosphorylation at Ser187 regulates synaptic facilitation and

short-term plasticity in an age-dependent manner, Sci. Rep. (2017). doi:10.1038/s41598-017-08237-x.

[141] J. Gao, M. Hirata, A. Mizokami, J. Zhao, I. Takahashi, H. Takeuchi, M. Hirata, Differential role of SNAP-25 phosphorylation by protein kinases A and C in the regulation of SNARE complex formation and exocytosis in PC12 cells., Cell. Signal. (2016). doi:10.1016/j.cellsig.2015.12.014.

[142] Y. Shimazaki, T.I. Nishiki, A. Omori, M. Sekiguchi, Y. Kamata, S. Kozaki, M. Takahashi, Phosphorylation of 25-kDa synaptosome-associated protein: Possible involvement in protein kinase C-mediated regulation of neurotransmitter release, J. Biol. Chem. (1996). doi:10.1074/jbc.271.24.14548.

[143] E. Mura, S. Preda, S. Govoni, C. Lanni, L. Trabace, M. Grilli, F. Lagomarsino, A. Pittaluga, M. Marchi , Specific neuromodulatory actions of amyloid-beta on dopamine release in rat nucleus accumbens and caudate putamen, J Alzheimers Dis. 19(3):1041 (2010). doi:doi: 10.3233/JAD-2010-1299.

[144] J.P. Tyszkiewicz, β-Amyloid Peptides Impair PKC-Dependent Functions of Metabotropic Glutamate Receptors in Prefrontal Cortical Neurons, J. Neurophysiol. (2005). doi:10.1152/jn.00939.2004.

[145] P. Zhong, Z. Gu, X. Wang, H. Jiang, J. Feng, Z. Yan, Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease, J.Biol.Chem. (2003). doi:10.1074/jbc.M302789200.

[146] P. De Camilli, R. Jahn, Pathways to Regulated Exocytosis in Neurons, Annu. Rev. Physiol. (1990). doi:10.1103/PhysRevLett.65.2362.

[147] K.M. Turner, R.D. Burgoyne, A. Morgan, Protein phosphorylation and the regulation of synaptic membrane traffic, Trends Neurosci. (1999). doi:10.1016/S0166-2236(99)01436-8.

[148] I. Ninan, O. Arancio, Presynaptic CaMKII is necessary for synaptic plasticity in cultured hippocampal neurons, Neuron. (2004). doi:10.1016/S0896-6273(04)00143-6.

[149] Y. Watanabe, N. Katayama, K. Takeuchi, T. Togano, R. Itoh, M. Sato, M. Yamazaki, M. Abe, T. Sato, K. Oda, M. Yokoyama, K. Takao, M. Fukaya, T. Miyakawa, M. Watanabe, K. Sakimura, T. Manabe, M. Igarashia, Point mutation in syntaxin-1A causes abnormal vesicle recycling, behaviors, and short term plasticity, J. Biol. Chem. (2013). doi:10.1074/jbc.M113.504050.

[150] K. Bodhinathan, A. Kumar, T.C. Foster, Intracellular Redox State Alters NMDA Receptor Response during Aging through Ca2+/Calmodulin-Dependent Protein Kinase II, J. Neurosci. (2010). doi:10.1523/JNEUROSCI.5485-09.2010.

[151] N. Amada, K. Aihara, R. Ravid, M. Horie, Reduction of NRI and phosphorylated Ca2+/calmodulin-dependent protein kinase II levels in Alzheimer's disease, Neuroreport. (2005). doi:10.1097/01.wnr.0000185015.44563.5d.

[152] R. Killick, E.M. Ribe, R. Al-Shawi, B. Malik, C. Hooper, C. Fernandes, R. Dobson, P.M. Nolan, A. Lourdusamy, S. Furney, K. Lin, G. Breen, R. Wroe, A.W.M. To, K. Leroy, M. Causevic, A. Usardi, M. Robinson, W. Noble, R. Williamson, K. Lunnon, S. Kellie, C.H. Reynolds, C. Bazenet, A. Hodges, J.P. Brion, J. Stephenson, J. Paul Simons, S. Lovestone, Clusterin regulates β-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway, Mol. Psychiatry. (2014). doi:10.1038/mp.2012.163.

[153] S.O. Yoon, D.J. Park, J.C. Ryu, H.G. Ozer, C. Tep, Y.J. Shin, T.H. Lim, L. Pastorino, A.J. Kunwar, J.C. Walton, A.H. Nagahara, K.P. Lu, R.J. Nelson, M.H. Tuszynski, K. Huang, JNK3 Perpetuates Metabolic Stress Induced by Aβ Peptides, Neuron. (2012). doi:10.1016/j.neuron.2012.06.024.

[154] S. Biggi, L. Buccarello, A. Sclip, P. Lippiello, N. Tonna, C. Rumio, D. Di Marino, M.C. Miniaci, T. Borsello, Evidence of Presynaptic Localization and Function of the c-Jun N-Terminal Kinase, Neural Plast. (2017). doi:10.1155/2017/6468356.

[155] R. Lalonde, K. Fukuchi, C. Strazielle, APP transgenic mice for modelling behavioural and psychological symptoms of dementia (BPSD), Neurosci. Biobehav. Rev. (2012). doi:10.1016/j.neubiorev.2012.02.011.

[156] M.I. Geerlings, B. Schmand, A.W. Braam, C. Jonker, L.M. Bouter, W. Van Tilburg, Depressive symptoms and risk of Alzheimer's disease in more highly educated older people, J. Am. Geriatr. Soc. (2000). doi:10.1111/j.1532-5415.2000.tb04785.x.

[157] M.G. Morgese, S. Schiavone, L. Trabace, Emerging role of amyloid beta in stress response: Implication for depression and diabetes, Eur. J. Pharmacol. (2017). doi:10.1016/j.ejphar.2017.08.031.

[158] M. Colaianna, P. Tucci, M. Zotti, M. Morgese, S. Schiavone, S. Govoni, V. Cuomo, L. Trabace, Soluble β-amyloid 1-42: A critical player in producing behavioural and biochemical changes evoking depressive-related state?, Br. J. Pharmacol. (2010). doi:10.1111/j.1476-5381.2010.00669.x.

[159] R.S. Filali M, Lalonde R, Cognitive and non-cognitive behaviors in an APPswe/PS1 bigenic model of Alzheimer's disease, Genes Brain Behav. 8(2):143-8 (2009).

[160] V. Krishnan, E.J. Nestler, The molecular neurobiology of depression, Nature. (2008). doi:10.1038/nature07455.

[161] D. Van Dam, Y. Vermeiren, A. D. Dekker, P. J.W. Naudé, P. P. De Deyn, Neuropsychiatric Disturbances in Alzheimer's Disease: What Have We Learned from Neuropathological Studies?, Curr. Alzheimer Res. (2016). doi:10.2174/1567205013666160502123607.

[162] P.B. Rosenberg, M.M. Mielke, B.S. Appleby, E.S. Oh, Y.E. Geda, C.G. Lyketsos, The association of neuropsychiatric symptoms in MCI with incident dementia and alzheimer disease, Am. J. Geriatr. Psychiatry. (2013). doi:10.1016/j.jagp.2013.01.006.

[163] N.J. Donovan, R.E. Amariglio, A.S. Zoller, R.K. Rudel, T. Gomez-Isla, D. Blacker, B.T. Hyman, J.J. Locascio, K.A. Johnson, R.A. Sperling, G.A. Marshall, D.M. Rentz, Subjective cognitive concerns and neuropsychiatric predictors of progression to the early clinical stages of Alzheimer disease, Am. J. Geriatr. Psychiatry. (2014). doi:10.1016/j.jagp.2014.02.007.

[164] F.E. Taragano, R.F. Allegri, C. Lyketsos, Mild behavioral impairment A prodromal stage of dementia, Dement. Neuropsychol. (2008). doi:10.1590/S1980-57642009DN20400004.

[165] J. Cummings, Chung JA, Neurobehavioral and neuropsychiatric symptoms in Alzheimer's disease, Neurol Clin. 18,829–846 (2000).

[166] H.C. Guimarães, R. Levy, A.L. Teixeira, R.G. Beato, P. Caramelli, Neurobiology of apathy in Alzheimer's disease, Arq. Neuropsiquiatr. (2008). doi:10.1590/S0004-282X2008000300035.

[167] C.J. Marshall GA, Fairbanks LA, Tekin S, Vinters HV, Neuropathological correlates of apathy in Alzheimer's disease, Dement Geriatr Cogn Disord. 21:144–7 (2006).

[168] R.A. Mitchell, N. Herrmann, K.L. Lanctôt, The Role of Dopamine in Symptoms and Treatment of Apathy in Alzheimer's Disease, CNS Neurosci. Ther. (2011). doi:10.1111/j.1755-5949.2010.00161.x.

[169] D. Storga, K. Vrecko, J.G.D. Birkmayer, G. Reibnegger, Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients, Neurosci. Lett. (1996). doi:10.1016/0304-3940(95)12256-7.

[170] R.A. Sweet, R.L. Hamilton, M.T. Healy, S.R. Wisniewski, R. Henteleff, B.G. Pollock, D.A. Lewis, S.T. DeKosky, Alterations of striatal dopamine receptor binding in Alzheimer disease are associated with Lewy body pathology and antemortem psychosis, Arch. Neurol. (2001). doi:10.1001/archneur.58.3.466.

[171] M. Benoit, I. Dygai, O. Migneco, P.H. Robert, C. Bertogliati, J. Darcourt, J. Benoliel, V. Aubin-Brunet, D. Pringuey, Behavioral and psychological symptoms in Alzheimer's disease. Relation between apathy and regional cerebral perfusion, Dement. Geriatr. Cogn. Disord. (1999). doi:10.1159/000017198.

[172] P.H. Robert, G. Darcourt, M.P. Koulibaly, S. Clairet, M. Benoit, R. Garcia, O. Dechaux, J. Darcourt, Lack of initiative and interest in Alzheimer's disease: A single photon emission computed tomography study, Eur. J. Neurol. (2006). doi:10.1111/j.1468-1331.2006.01088.x.

[173] P.D. Bruen, W.J. McGeown, M.F. Shanks, A. Venneri, Neuroanatomical correlates of neuropsychiatric symptoms in Alzheimer's disease, Brain. (2008). doi:10.1093/brain/awn151.

[174] V.A. Holthoff, B. Beuthien-Baumann, E. Kalbe, S. Lüdecke, O. Lenz, G. Zündorf, S. Spirling, K. Schierz, P. Winiecki, S. Sorbi, K. Herholz, Regional cerebral metabolism in early Alzheimer's disease with clinically significant apathy or depression, Biol. Psychiatry. (2005). doi:10.1016/j.biopsych.2004.11.035.

[175] G.A. Marshall, L. Monserratt, D. Harwood, M. Mandelkern, J.L. Cummings, D.L. Sultzer, Positron emission tomography metabolic correlates of apathy in Alzheimer disease, Arch. Neurol. (2007). doi:10.1001/archneur.64.7.1015.

[176] M. Benoit, G. Berrut, J. Doussaint, S. Bakchine, S. Bonin-Guillaume, P. Frémont, T. Gallarda, P. Krolak-Salmon, T. Marquet, C. Mékiès, F. Sellal, S. Schuck, R. David, P. Robert , Apathy and depression in mild Alzheimer's disease: a cross-sectional study using diagnostic criteria, J Alzheimers Dis. 31(2):325- (2012). doi:doi: 10.3233/JAD-2012-112003.

[177] R.J. Mourao, G. Mansur, L.F. Malloy-Diniz, E. Castro Costa, B.S. Diniz, Depressive symptoms increase the risk of progression to dementia in subjects with mild cognitive impairment: systematic review and meta-analysis, Int. J. Geriatr. Psychiatry. (2016). doi:10.1002/gps.4406.

[178] T. Leyhe, C.F. Reynolds, T. Melcher, C. Linnemann, S. Klöppel, K. Blennow, H. Zetterberg, B. Dubois, S. Lista, H. Hampel, A common challenge in older adults:

Classification, overlap, and therapy of depression and dementia, Alzheimer's Dement. (2017). doi:10.1016/j.jalz.2016.08.007.

[179] Y. Vermeiren, D. Van Dam, T. Aerts, S. Engelborghs, P.P. De Deyn, Brain region-specific monoaminergic correlates of neuropsychiatric symptoms in Alzheimer's disease, J. Alzheimers. Dis. (2014). doi:10.3233/JAD-140309.

[180] L. Trillo, D. Das, W. Hsieh, B. Medina, S. Moghadam, B. Lin, V. Dang, M.M. Sanchez, Z. De Miguel, J.W. Ashford, A. Salehi, Ascending monoaminergic systems alterations in Alzheimer's disease. Translating basic science into clinical care, Neurosci. Biobehav. Rev. (2013). doi:10.1016/j.neubiorev.2013.05.008.

[181] F. Yasuno, H. Kazui, N. Morita, K. Kajimoto, M. Ihara, A. Taguchi, A. Yamamoto, K. Matsuoka, J. Kosaka, T. Kudo, H. Iida, T. Kishimoto, K. Nagatsuka, High amyloid-β deposition related to depressive symptoms in older individuals with normal cognition: a pilot study, Int. J. Geriatr. Psychiatry. (2016). doi:10.1002/gps.4409.

[182] C. Ballard, A. Corbett, Agitation and aggression in people with Alzheimer's disease, Curr. Opin. Psychiatry. (2013). doi:10.1097/YCO.0b013e32835f414b.

[183] L.C. Rosenberg PB, Nowrangi MA, Neuropsychiatric symptoms in Alzheimer's disease: what might be associated brain circuits?, Mol Asp. Med. 43–44:25–3 (2015).

[184] A.M. Palmer, G.C. Stratmann, A.W. Procter, D.M. Bowen, Possible neurotransmitter basis of behavioral changes in alzheimer's disease, Ann. Neurol. (1988). doi:10.1002/ana.410230616.

[185] A. Russo-Neustadt, T.J. Zomorodian, C.W. Cotman, Preserved cerebellar tyrosine hydroxylase-immunoreactive neuronal fibers in a behaviorally aggressive subgroup of Alzheimer's disease patients, Neuroscience. (1998). doi:10.1016/S0306-4522(98)00134-1.

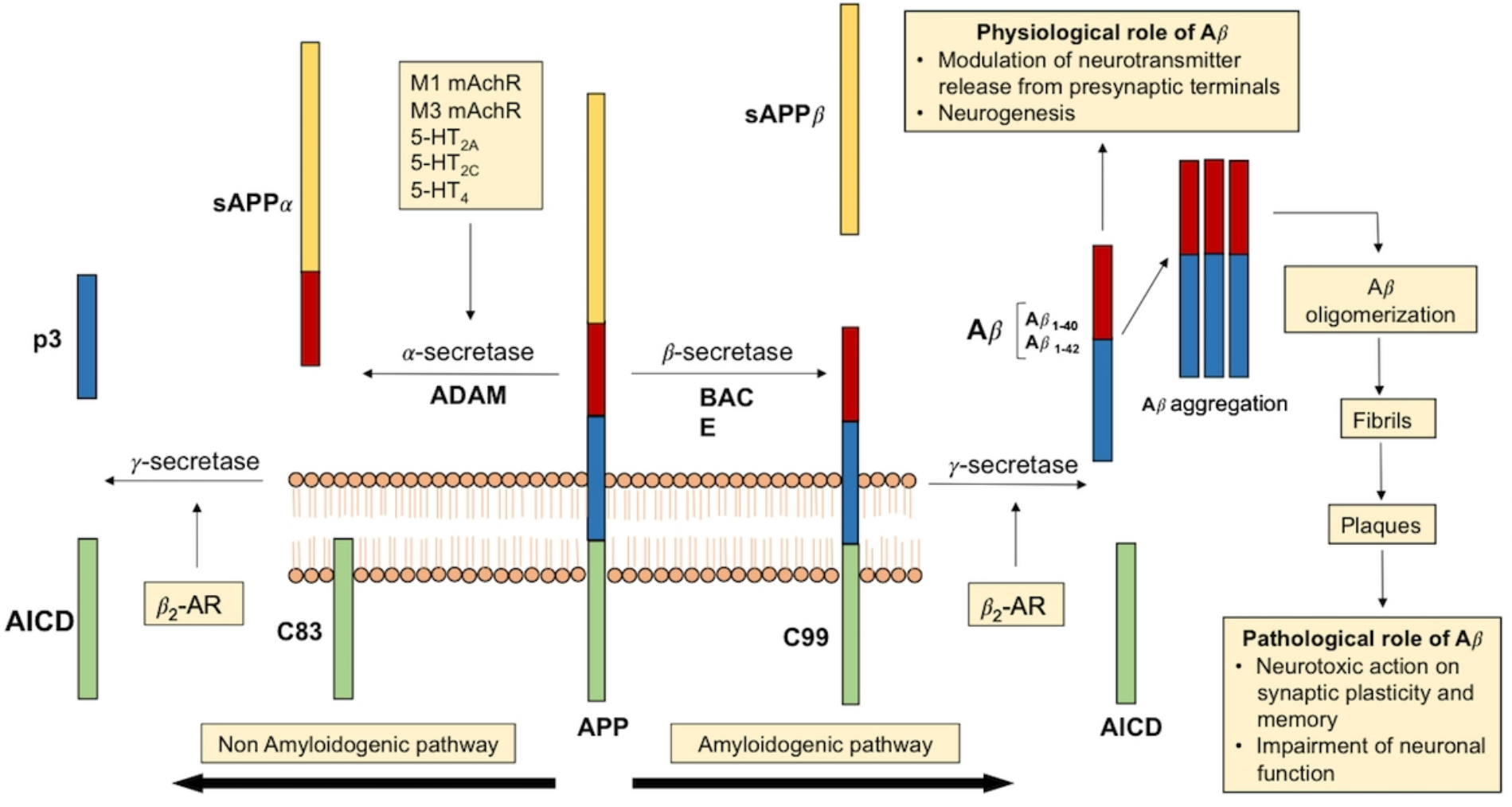
[186] J.L. Cummings, T. McRae, R. Zhang, Effects of donepezil on neuropsychiatric symptoms in patients with dementia and severe behavioral disorders, Am. J. Geriatr. Psychiatry. (2006). doi:10.1097/01.JGP.0000221293.91312.d3.

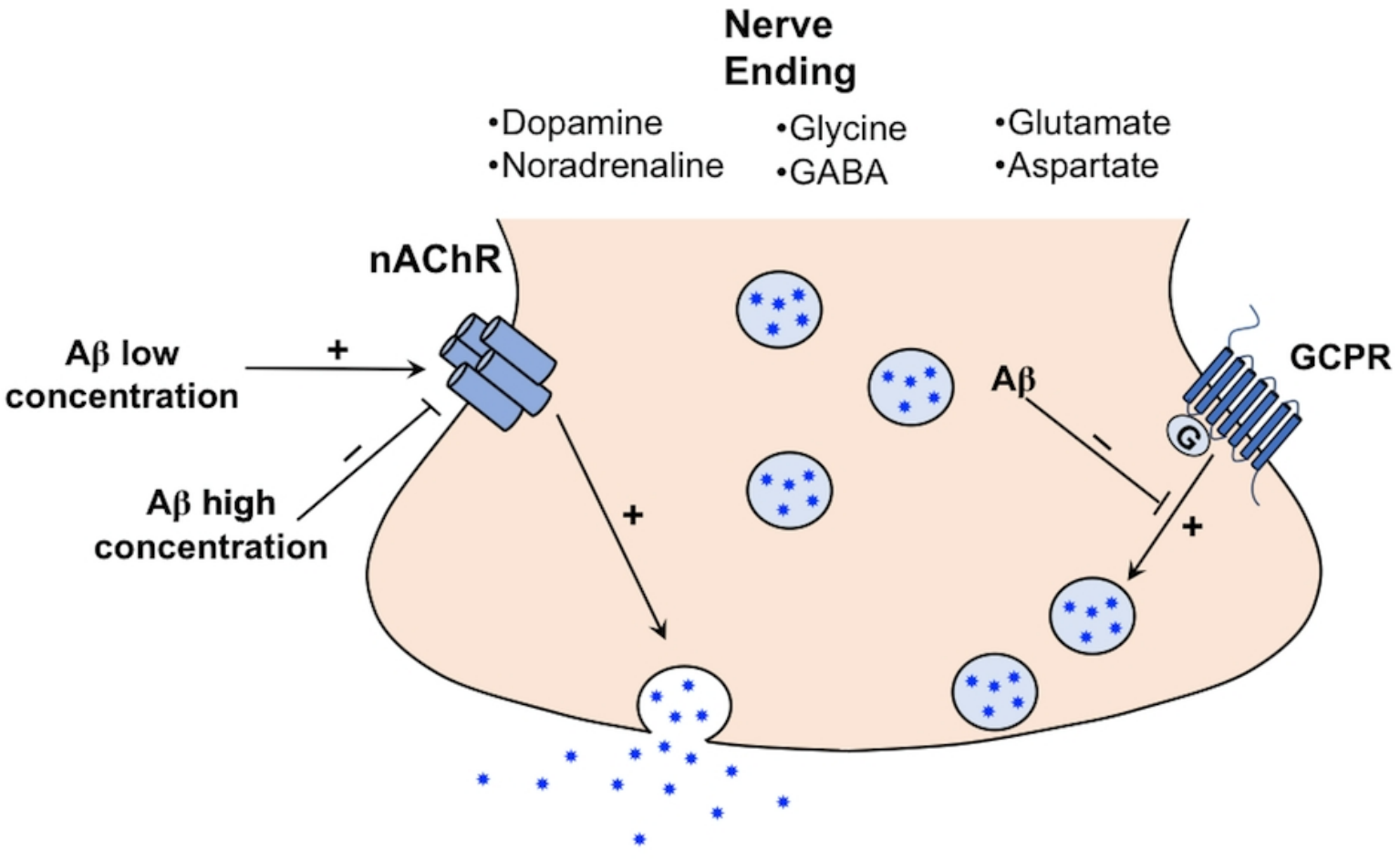
[187] L.C. Leroi I, Voulgari A, Breitner JC, The epidemiology of psychosis in dementia, Am J Geriatr Psychiatry. 11:83–91 (2003).

[188] S.H. Lin, C.Y. Yu, M.C. Pai, The occipital white matter lesions in Alzheimer's disease patients with visual hallucinations, Clin. Imaging. (2006). doi:10.1016/j.clinimag.2006.09.025.

[189] L. Serra, R. Perri, M. Cercignani, B. Spanò, L. Fadda, C. Marra, G.A. Carlesimo, C. Caltagirone, M. Bozzali, Are the behavioral symptoms of Alzheimer's disease directly associated with neurodegeneration?, J. Alzheimer's Dis. (2010). doi:10.3233/JAD-2010-100048.

[190] G.S. Zubenko, J. Moossy, A.J. Martinez, G. Rao, D. Claassen, J. Rosen, U. Kopp, Neuropathologic and Neurochemical Correlates of Psychosis in Primary Dementia, Arch. Neurol. (1991). doi:10.1001/archneur.1991.00530180075020.


Physiological role of Aβ
• Modulation of neurotransmitter release from presynaptic terminals
• Neurogenesis
M1 mAchR
M3 mAchR
5-HT2A
5-HT2C
5-HT4
sAPPα
sAPPβ
p3
α-secretase
ADAM
β-secretase
BACE
Aβ
Aβ 1-40
Aβ 1-42
Aβ aggregation
Aβ oligomerization
Fibrils
Plaques
γ-secretase
γ-secretase
AICD
β2-AR
β2-AR
C83
C99
APP
AICD
Non Amyloidogenic pathway
Amyloidogenic pathway
Pathological role of Aβ
• Neurotoxic action on synaptic plasticity and memory
• Impairment of neuronal function



Neurobehavioral rather than degenerative changes as a consequence of Aβ dysregulation

Table 1. Interactions between beta-amyloid and cholinergic receptors in regulating neurotransmitter release.

Beta-amyloid influence on cholinergic control of neurotransmitter release				
Molecular species of Aβ	**Aggregation status and concentration/time of exposure**	**Observed effect of beta-amyloid on neurotransmitters**	**Experimental models/brain area**	**Reference**
Dopamine				
Soluble $A\beta_{1-40}$ and $A\beta_{1-42}$	1-10 μM/60-80 min (for *in vivo* experiments); 100 nM/up to 10 min (for *in vitro* analysis)	- In rat nucleus accumbens, 1 μM soluble Aβ completely counteracted the muscarinic receptor-activated DA release in a reversible manner, whereas the overflow of DA elicited by nicotinic receptors activation through epibatidin administration was not altered by Aβ infusion. - The [^{3}H]DA release evoked by carbachol (30 mM) in accumbal isolated nerve endings is significantly reduced by 100 nM Aβ. Also $A\beta_{1-42}$ (100 nM) significantly reduced the DA release evoked by carbachol to a similar extent.	*In vivo* (brain dialysis) and *in vitro* (isolated synaptosomes) models/Rat nucleus accumbens	[46]
$A\beta_{1-40}$	100 nM	The extracellular application of $A\beta_{1-40}$ (100 nM) inhibited both nicotinic and muscarinic cholinergic modulation of DA release by acting respectively from outside and inside the nerve endings.	Synaptosomes/ Rat nucleus accumbens	[82]
$A\beta_{1-40}$ and $A\beta_{1-42}$	10-100 nM/up to 12 min	Experiments on isolated nerve endings: - Aβ impaired the muscarinic control of DA release in both nucleus accumbens and caudate putamen; - Aβ affected a specific component of the DA overflow evoked by the non-selective metabotropic glutamate receptors agonist t-ACPD in caudate putamen	Synaptosomes/Caudate-putamen-Nucleus Accumbens	[143]
GABA				
$A\beta_{1-40}$ and $A\beta_{1-42}$	100 nM/up to 17 min	Experiments on isolated nerve endings: - Aβ inhibited GABA release selectively acting on muscarinic receptor subtypes which stimulate transmitter release (M3 and M5); - Aβ was ineffective on muscarinic receptor subtypes which modulate negatively the stimulated transmitter release (M2 and M4).	Synaptosomes/ Rat nucleus accumbens	[45]

Monomers of $A\beta_{1-40}$	100 nM,1 μM,10 μM/40-60 min (for *in vivo* experiments); 100 pM, 1 nM, 100 nM/up to 10 min (for *in vitro* analysis)	- Perfusion of 10 μM Aβ (microdialysis) inhibited the nicotine-induced release of GABA; - Perfusion of 100 nM Aβ (microdialysis) potentiated the nicotine-evoked GABA overflow; - Experiments on isolated nerve endings: 100 nM Aβ inhibited the nicotine-induced release of GABA; 100 nM Aβ inhibited the release of GABA induced by the α4β2 selective agonist 5IA85380.	*In vivo* (microdialysis) and *in vitro* (synaptosomes in superfusion) techniques/Hippocampus	[48]
Glycine				
$A\beta_{1-40}$	10 μM/40-60 min (for *in vivo* experiments); 10 nM, 100 nM/up to 10 min (for *in vitro* analysis)	- Perfusion of 10 μM $A\beta_{1-40}$ (microdialysis) reduced the nicotine-induced Gly overflow and also the Gly overflow induced by the α7 selective agonist PHA543613; - Experiments on isolated nerve endings: both 10 nM and 100 nM Aβ inhibited the nicotine-induced Gly release; 100 nM Aβ inhibited the release of Gly evoked by the α7 selective agonist carbachol and by the α4β2 selective agonist 5IA85380.	*In vitro* (synaptosomes in superfusion) and *in vivo* (microdialysis) approaches/Hippocampus	[49]
Aspartate				
Monomers of $A\beta_{1-40}$	100 nM,1 μM,10 μM/40-60 min (for *in vivo* experiments); 100 pM 1 nM 100 nM/up to 10 min (for *in vitro* analysis)	- Perfusion of 10 μM and 1 μM Aβ (microdialysis) inhibited the nicotine-induced release of aspartate; - Experiments on isolated nerve endings: 100 nM Aβ inhibited the nicotine-induced release of aspartate; 100 nM Aβ inhibited the release of aspartate that was induced by the α7 selective agonist carbachol; 100 nM Aβ inhibited the release of aspartate induced by the α4β2 selective agonist 5IA85380; 100 pM Aβ potentiated the carbachol-induced release of aspartate.	*In vivo* (microdialysis) and *in vitro* (synaptosomes in superfusion) techniques/Hippocampus	[48]
Glutamate				
Monomers of $A\beta_{1-40}$	100 nM,1 μM,10 μM/40-60 min (for *in vivo* experiments); 100 pM, 1 nM, 100 nM/up to 10 min (for *in vitro* analysis)	- Perfusion of 10 μM and 1 μM Aβ (microdialysis) inhibited the nicotine-induced release of glutamate; - Experiments on isolated nerve endings: 100 nM Aβ inhibited the nicotine-induced release of glutamate; 100 nM Aβ inhibited the release of glutamate induced by the α7 selective agonist carbachol; 1 nM Aβ potentiated the release of	*In vivo* (microdialysis) and *in vitro* (synaptosomes in superfusion) techniques/Hippocampus	[48]

		glutamate induced by carbachol; 100 nM Aβ inhibited the release of glutamate induced by the α4β2 selective agonist 5IA85380; 100 pM Aβ potentiated the carbachol-induced release of glutamate.		

Table 2. Beta-amyloid induced dysfunctions at the level of synapses of different neurotransmitter.

Molecular species of Aβ	Aggregation status and concentration/time of exposure	Observed effect of beta-amyloid on neurotransmitters	Experimental models/ brain areas	Reference
Noradrenergic system				
Freshly prepared $A\beta_{1-42}$	4 μM	- A single i.c.v. injection of $A\beta_{1-42}$ solution (4 μM) induced a significant increase of NE concentrations in the prefrontal cortex, nucleus accumbens and hippocampus, 2 h after the administration.	Single i.c.v. injection of $A\beta_{1-42}$ in rats/prefrontal cortex, nucleus accumbens and hippocampus	[71]
Dopaminergic system				
Soluble $A\beta_{1-42}$	4 μM	- In the prefrontal cortex a single i.c.v. injection of soluble $A\beta_{1-42}$ (4 μM) induced a marked reduction in extracellular concentrations of basal DA, when measured 2 h and 2 days after peptide administration. - The increase in DA release stimulated by local 100 mM K perfusion was abolished in $A\beta_{1-42}$-injected rats.	Single i.c.v. injection of $A\beta_{1-42}$ in rats/prefrontal cortex	[83]
Serotoninergic system				
Soluble $A\beta_{1-42}$	4 μM	- Soluble $A\beta_{1-42}$ peptide (4 μM) has been described to selectively reduce 5-HT content and BDNF expression of either its mRNA or protein in the rat prefrontal cortex.	Injection of $A\beta_{1-42}$ in rats/prefrontal cortex	[158]

Table 3. Direct interplay between beta-amyloid and SNARE proteins.

Molecular species of Aβ	Aggregation status and concentration/time of exposure	Observed effect on SNARE proteins	*In vitro* and *in vivo* model	Reference
Syntaxin 1a				
$A\beta_{1-40}$ and $A\beta_{1-42}$	Both monomers and oligomers (10 μM)	- Oligomeric form of Aβ (10μM) has been found to exert an inhibitory effect on SNARE-mediated exocytosis by binding to the SNARE motif region (SynH3) of Syntaxin 1a, thus specifically inhibiting the fusion step between docking and lipid mixing. - Aβ monomers failed to exhibit any inhibitory effects on SNARE complex formation or membrane fusion, despite their proved capability to bind to SynH3 of Syntaxin 1a.	*In vitro* single-vesicle content-mixing assay	[128]
Synaptophysin/VAMP complex				
$A\beta_{1-42}$	Monomers (50 nM/20 min)	- Aβ42 has been demonstrated to directly compete with VAMP2 for binding Synaptophysin at synaptic contacts, thus preventing the formation of Synaptophysin/VAMP complex and, subsequently, inducing the formation of the fusion pore complex followed by neurotransmitter release. - Electrophysiology recordings in brain slices confirmed that Aβ42 affects baseline transmission, by preventing the formation of Synaptophysin/VAMP complex. Indeed, in hippocampal slices, the enhancement of single-shock fEPSPs by Aβ42 at synapses further suggest an increased availability of releasable synaptic vesicles.	Primary cultures of CA3-CA1 rat hippocampal neurons	[131]

UNIVERSITÀ DI PAVIA
Dipartimento di Scienze del Farmaco
Università degli Studi di Pavia

AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Signed by all authors as follows:

Cristina Lanni

Francesca Fagiani

Marco Racchi

Stefania Preda

Alessia Pascale

Massimo Grilli

Nicola Allegri 

Stefano Govoni