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Lennart Mucke and Dennis J. Selkoe

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Neurotoxicity of Amyloid β -Protein: Synaptic and Network Dysfunction

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Evidence for an ever-expanding variety of molecular mediators of amyloid β -protein neurotoxicity (membrane lipids, receptor proteins, channel proteins, second messengers and related signaling cascades, cytoskeletal proteins, inflammatory mediators, etc.) has led to the notion that the binding of hydrophobic $A\beta$ assemblies to cellular membranes triggers multiple effects affecting diverse pathways. It appears unlikely that there are only one or two cognate receptors for neurotoxic forms of $A\beta$ and also that there are just one or two assembly forms of the peptide that induce neuronal dysfunction. Rather, various soluble (diffusible) oligomers of $A\beta$ that may be in dynamic equilibrium with insoluble, fibrillar deposits (amyloid plaques) and that can bind to different components of neuronal and non-neuronal plasma membranes appear to induce complex patterns of synaptic dysfunction and network disorganization that underlie the intermittent but gradually progressive cognitive manifestations of the clinical disorder. Modern analyses of this problem utilize electrophysiology coupled with synaptic biochemistry and behavioral phenotyping of animal models to elucidate the affected circuits and assess the effects of potential therapeutic interventions.

A quarter of a century of research on amyloid β -protein ($A\beta$) has produced a wealth of evidence that its accumulation in brain regions serving memory and cognition contributes strongly to the development of Alzheimer disease (AD). Support has come from neuropathological, genetic, biochemical, animal modeling, biomarker and, recently, therapeutic studies. There is now little doubt that the accumulation of certain forms of $A\beta$ is associated with, and probably induces, profound neuronal changes

in the brain. Cells other than neurons, including microglia, astrocytes, and the endothelial and smooth muscle cells of cerebral blood vessels, can also be altered functionally and structurally by excessive $A\beta$ levels. However, it is generally assumed that adverse effects of $A\beta$ specifically on neurons and their processes help initiate the cardinal memory and cognitive deficits that define AD. The precise biochemical mechanisms by which various assembly forms of the peptide cause neuronal

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dysfunction and ultimately death remain to be defined.

Our focus in this chapter is the neuron and, in particular, the synapse. We emphasize that numerous synaptic and nonsynaptic neuronal changes, as well as effects on cells other than neurons, are likely to occur virtually simultaneously as the disease develops and progresses. Accordingly, it is simplistic to think about the actions of A β on neurons—both individually and in networks—in the absence of the non-neuronal events (e.g., microgliosis, astrogliosis, microvascular injury) that could contribute to altered neuronal integrity and function secondarily. Nevertheless, we will dissect this remarkably complex scenario in a reductionist fashion, focusing first and foremost on synaptic/neuronal changes induced by A β ; these changes must ultimately be integrated with the effects on other cell types described in other articles in this collection.

MONOMERS, OLIGOMERS, AND FIBRILS: CHANGING IDEAS ABOUT WHICH FORMS OF A β IMPAIR NEURONAL FUNCTION AND HOW THEY DO SO

Early versions of the amyloid cascade hypothesis of AD posited adverse effects of amyloid plaques on surrounding dendrites, axons and glia, based in part on the light microscopic appearance of neuritic plaques (Selkoe 1991; Hardy and Higgins 1992). However, the recognition of buffer-soluble bioactive oligomers (e.g., dimers, trimers, tetramers, dodecamers, higher oligomers) in synthetic A β peptide preparations (Lambert et al. 1998; Bitan et al. 2001; Kaye et al. 2003), in cell culture media (Podlisny et al. 1995; Walsh et al. 2002), in amyloid precursor protein (APP) transgenic mouse brains (Kawarabayashi et al. 2001; Lesne et al. 2006; Shankar et al. 2009), and in AD brain tissue (Roher et al. 1996; McLean et al. 1999; Gong et al. 2003; Shankar et al. 2008) gave rise to the concept that the insoluble amyloid fibrils comprising the plaques might themselves be relatively inactive but serve as reservoirs of these smaller, potentially neurotoxic assemblies. Similarly, protofibrils of synthetic A β that were

thinner than classical 8 nm amyloid fibrils could be generated from synthetic A β peptide under certain in vitro conditions and also induce neurotoxic effects (Harper et al. 1997; Walsh et al. 1997; Hartley et al. 1999). These biochemical findings, coupled with analogous experimental observations for other pathogenic neuronal proteins (e.g., huntingtin and α -synuclein), have increasingly led the field to consider small, readily diffusible assemblies as principal cytotoxic forms of misfolded, self-aggregating proteins. The concept is consistent with—and emerged in part from—the demonstration that APP transgenic mice show electrophysiological, neuroanatomical and behavioral abnormalities well before the appearance of microscopically visible A β deposits (Holcomb et al. 1999; Hsia et al. 1999; Mucke et al. 2000).

This modification of the so-called “amyloid hypothesis” based on new findings does not rule out a neurotoxic role for amyloid plaques themselves. Indeed, there is abundant evidence of neuritic alteration in the immediate vicinity of AD plaques, such as local distortion and curvature of normally rather straight cortical dendrites around plaques, raising the possibility of decreased efficiency of neurotransmission along them (Hyman et al. 1995). Moreover, in APP transgenic mice, array tomography has revealed a striking penumbra of excitatory synapse loss and neuritic dystrophy that is greatest immediately adjacent to a plaque and lessens in a radial fashion, becoming virtually normal approximately 30–50 μ m away from the plaque core edge (Spires-Jones et al. 2007; Koffie et al. 2009). Somewhat analogous findings have been described in sections of AD cortex (Serrano-Pozo et al. 2010). In mice, this penumbra is reactive with antibodies (e.g., Nab61) that are relatively specific for A β oligomers, at least in immunochemical assays. Although it is possible that such antibodies do not retain their oligomer specificity in the complex epitope environment of brain sections, such morphological analyses suggest that plaques confer synaptic and neuritic effects in part by acting as local reservoirs of diffusible oligomers. Independent experiments in which soluble oligomers and insoluble amyloid plaque cores were

biochemically isolated from the same AD cortices and assayed electrophysiologically on wild-type mouse brain slices showed that soluble oligomers potently blocked LTP, whereas washed amyloid cores did not, unless they were first dissolved in harsh solvents (e.g., formic acid) to release their constituent oligomers (Shankar et al. 2008). In this context, it has been found that lipids can convert inert A β amyloid fibrils into neurotoxic protofibrils that can then alter learning in mice (Martins et al. 2008). Taken together, these and other experimental approaches suggest that plaques may confer local neurotoxicity because they are in equilibrium with surrounding oligomers and protofibrils. In principle, it makes biophysical sense that small oligomers would be more synaptotoxic than plaques, as the former collectively provide a much greater surface area for interaction with neurons (and glia) and their processes than do the large, nondiffusible plaques.

EARLY VERSUS LATE: REFOCUSING THE INVESTIGATIVE EMPHASIS FROM FRANK NEURODEGENERATION ONTO EARLIER SYNAPTIC PERTURBATIONS CAUSED BY A β

Most mouse lines transgenic for human (h) APP do not show overt neuronal loss, and this aspect of their phenotype is often criticized as a weakness of these models. However, it is unknown whether the loss of neurons in AD brains is directly caused by A β accumulation and, even if it is, whether it takes A β less than 2–3 years (the typical lifetime of a mouse) to kill neurons in the human brain. The notion that hAPP transgenic mice do not undergo neurodegeneration is a misunderstanding, in that they do develop substantial neuritic dystrophy and synapse loss, which are clear signs of a neuronal degenerative process, even if counts of cell bodies are not significantly decreased. Thus, hAPP mice are good models of A β -induced synaptic dysfunction. For the following reasons, this feature alone makes them directly relevant to the human condition.

Some two decades ago, quantitative neuropathological analyses revealed strong associations between the degrees of cognitive impairment and

synaptic alteration in AD subjects (DeKosky and Scheff 1990; Terry et al. 1991). Subsequent studies in hAPP transgenic mice and other experimental systems demonstrated that A β oligomers modulate both pre- and postsynaptic structures and functions in a dose- and assembly-dependent manner (for reviews, see Selkoe 2002; Palop and Mucke 2010). In hAPP mice, manipulations that prevent or reverse synaptic deficits also prevent or reverse cognitive impairment (e.g., McLaurin et al. 2006; Cisse et al. 2011a; Roberson et al. 2011), supporting the hypothesis that A β causes cognitive deficits in part by interfering with synaptic functions. Because these hAPP mice have little overt neuronal loss and develop their synaptic and cognitive impairments before forming amyloid plaques, it is likely that their synaptic deficits are caused by soluble A β assemblies rather than by plaques per se, and that these deficits reflect primary synaptotoxicity rather than secondary consequences of neuronal degeneration. Consistent with this notion, synthetic A β oligomers and soluble A β oligomers isolated from cell culture media or AD brain extracts acutely impair synaptic functions when added to hippocampal slices or slice cultures (e.g., Gong et al. 2003; Shankar et al. 2007, 2008; Li et al. 2009). Collectively, these and many other studies in the last few years have refocused the experimental approach to A β neurotoxicity from frank cell death to more subtle structural and functional deficits of synapses and neurites.

MODULATION OF SYNAPTIC TRANSMISSION BY A β : A NEGATIVE REGULATOR OF NEURONAL ACTIVITY POSTSYNAPTICALLY, BUT A POTENTIAL POSITIVE REGULATOR PRESYNAPTICALLY

In vivo and in vitro studies have demonstrated that high levels of A β , particularly in oligomeric forms, alter glutamatergic synaptic transmission and cause synapse loss (Hsia et al. 1999; Mucke et al. 2000; Walsh et al. 2002; Kamenetz et al. 2003; Shankar et al. 2007; Li et al. 2009). On the other hand, the production of A β and its secretion into the extracellular space are regulated in part by neuronal activity in vitro (Kamenetz et al. 2003) and in vivo (Cirrito

et al. 2005). Increased neuronal activity enhances A β generation and blocking neuronal activity has the opposite effect (Kamenetz et al. 2003). This synaptic regulation of A β production is mediated, at least in part, by clathrin-dependent endocytosis of surface APP at presynaptic terminals, endosomal proteolytic cleavage of APP, and A β release at synaptic terminals (Cirrito et al. 2005). In addition, pathogenic A β species can also be released from dendrites (Wei et al. 2010). This neuronal activity-dependent regulation of A β secretion has been observed during pathological events, such as epileptiform activity induced by electrical stimulation (Cirrito et al. 2005), as well as during normal physiological processes, such as the sleep–wake cycle (Kang et al. 2009). Such experimental findings support the concept that APP, and its A β fragment in particular, are part of a feedback loop controlling neuronal excitability (Kamenetz et al. 2003). In this paradigm, A β production is enhanced by action potential-dependent synaptic activity, leading to increased levels of extracellular A β at and near synapses and reduction of excitatory transmission postsynaptically (Fig. 1). Pathologically elevated levels of A β would be expected to put this negative feedback regulator into overdrive, suppressing excitatory synaptic activity at the postsynaptic level. However, a caveat about some of the experimental observations just cited that underlie this model is that the investigators could not always be sure what assembly state the A β being detected was in, that is, soluble monomers and/or soluble oligomers. Because these assemblies are likely to exist in a dynamic equilibrium, it can be difficult to assign the neurophysiological effects of A β to a particular assembly form, depending on exactly how an experiment was conducted.

Some work suggests that A β could also act as a positive regulator at the presynaptic level. For example, relatively small increases in endogenous A β levels ($\sim 1.5\times$), induced by inhibition of extracellular A β degradation in otherwise unmanipulated wild-type neurons, enhanced the release probability of synaptic vesicles and increased neuronal activity in neuronal culture (Abramov et al. 2009). In this

study, enhanced extracellular A β increased spontaneous excitatory postsynaptic currents without significantly altering inhibitory currents. Importantly, all these effects were exclusively presynaptic and dependent on firing rates, with lower facilitation seen in neurons with higher firing rates. Thus, small increases of A β may facilitate presynaptic glutamatergic release in neurons with low activity but not in neurons with high activity. Generally consistent with the above findings, another study reported that application of low concentrations of synthetic A β 42 (picomolar range) markedly potentiated synaptic transmission, whereas higher concentrations of A β 42 (low nanomolar range) caused the expected synaptic depression (Puzzo et al. 2008). In this study, the potentiating effect of A β did not affect postsynaptic N-methyl D-aspartate receptor (NMDAR) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) currents but was dependent on α 7-nAChR activation, suggesting a presynaptic mechanism mediated by build-up of Ca²⁺ in presynaptic terminals. Thus, A β may directly act on presynaptic α 7-nAChR (Dineley et al. 2002) and be part of a positive feedback loop that increases presynaptic Ca²⁺ levels and A β secretion. Consistent with this model, blocking nAChRs or removing α 7-nAChRs decreased A β secretion and blocked A β -induced facilitation (Wei et al. 2010).

It should be pointed out that the interpretation of physiological experiments examining synthetic A β 42 is difficult, because its two extra hydrophobic residues (alanine and isoleucine) give it a remarkable propensity to aggregate, even at low concentrations. Oligomers of A β 42 should have different biological properties than monomers of A β 42 given their different structures. Consequently, in vitro studies of the normal function of A β should instead focus on the A β 40 peptide, as this is by far (tenfold) the most abundant A β monomer under physiological conditions in young mammals. Studies that attribute normal biological functions to low levels of A β 42 must confirm these findings using A β 40.

Another emerging lesson is that A β -induced presynaptic effects depend on an optimal

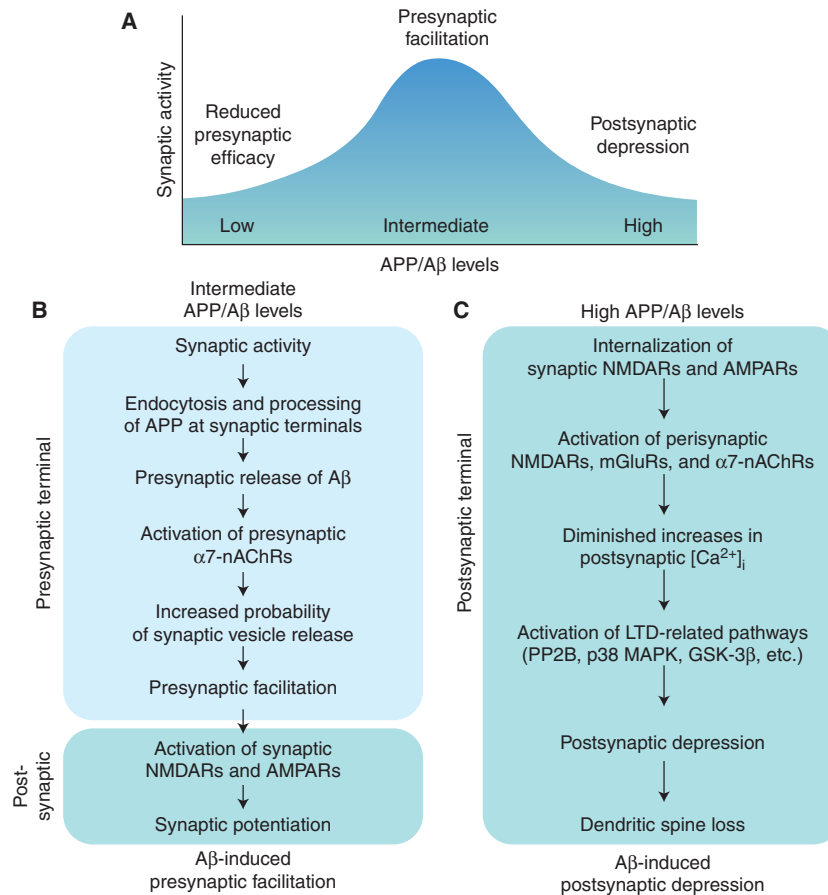


Figure 1. Presynaptic and postsynaptic regulation of synaptic transmission by amyloid β -protein ($A\beta$). (A) Hypothetical relationship between $A\beta$ level and synaptic activity. Intermediate levels of $A\beta$ enhance synaptic activity presynaptically, whereas abnormally high or low levels of $A\beta$ impair synaptic activity by inducing postsynaptic depression or reducing presynaptic efficacy, respectively. (B) Within a physiological range, small increases in $A\beta$ primarily facilitate presynaptic functions, resulting in synaptic potentiation. (C) At abnormally high levels, $A\beta$ enhances long-term depression (LTD)-related mechanisms, resulting in postsynaptic depression and loss of dendritic spines (modified from Palop and Mucke 2010).

$A\beta$ concentration (Fig. 1), with higher or lower concentrations potentially impairing synaptic transmission (Abramov et al. 2009). A positive modulatory effect of $A\beta$ on synaptic transmission is further supported indirectly by the finding that abnormally low levels of $A\beta$ in mice deficient for APP (Seabrook et al. 1999), PS1 (Saura et al. 2004), or BACE1 (Laird et al. 2005) are associated with synaptic transmission deficits. Overall, these and other data suggest an apparent bell-shaped relationship between extracellular $A\beta$ and synaptic transmission in

which intermediate levels of $A\beta$ potentiate presynaptic terminals, low levels reduce presynaptic efficacy, and high levels depress postsynaptic transmission.

Elevated Levels of $A\beta$ Impair Synaptic Transmission by Enhancing Synaptic Depression

Excitatory synaptic transmission is tightly regulated by the number of active NMDARs and AMPARs at the synapse. NMDAR activation

plays a central role, because it can induce either long-term potentiation (LTP) or long-term depression (LTD), depending on the extent of the resultant $[Ca^{2+}]_i$ rise in the dendritic spines and the downstream activation of specific intracellular cascades (Kullmann and Lamsa 2007). Activation of synaptic NMDARs and large increases in $[Ca^{2+}]_i$ are required for LTP, whereas internalization of synaptic NMDARs, activation of perisynaptic NMDARs, and lower increases in $[Ca^{2+}]_i$ are necessary for LTD. LTP induction promotes recruitment of AMPARs and growth of dendritic spines, whereas LTD induces spine shrinkage and synaptic loss (Kullmann and Lamsa 2007).

Pathological A β levels and assembly forms (e.g., oligomers) may indirectly cause a partial block of NMDARs and shift the activation of NMDAR-dependent signaling cascades toward pathways involved in the induction of LTD and synaptic loss (Fig. 1; Kamenetz et al. 2003; Hsieh et al. 2006; Shankar et al. 2007). This model is consistent with the fact that A β oligomers (but not monomers) impair LTP (Walsh et al. 2002; Shankar et al. 2008; Li et al. 2011) and enhance LTD (Fig. 1; Kim et al. 2001; Hsieh et al. 2006; Li et al. 2009). Although the mechanisms underlying A β -facilitated LTD have not yet been fully elucidated, they may involve receptor internalization (Snyder et al. 2005; Hsieh et al. 2006) or desensitization (Liu et al. 2004) and subsequent collapse of dendritic spines (Snyder et al. 2005; Hsieh et al. 2006). A β -dependent effects on synaptic function may be mediated by postsynaptic activation of $\alpha 7$ -nAChR (Snyder et al. 2005), activation of extrasynaptic NMDA receptors (Shankar et al. 2007; Li et al. 2009), and downstream effects on calcineurin/STEP/cofilin, p38 MAPK, and GSK-3 β signaling pathways, among others (Wang et al. 2004; Shankar et al. 2007; Li et al. 2009; Tackenberg and Brandt 2009).

Another way in which soluble A β oligomers may enhance LTD is by blocking neuronal glutamate uptake at synapses, leading to increased glutamate levels at the synaptic cleft (Fig. 2; Li et al. 2009). A resultant rise in glutamate levels would initially activate synaptic NMDARs

followed by desensitization of the receptors and, ultimately, synaptic depression. Another effect of increased glutamate levels would be a spillover and activation of extra- or perisynaptic NR2B-enriched NMDARs, which play a major role in LTD induction (Liu et al. 2004) and have also been shown to help mediate the inhibition of LTP by soluble A β oligomers (Li et al. 2011). The activation of perisynaptic receptors may thus be involved in the facilitation of LTD by A β and the inhibition of LTP (Hsieh et al. 2006; Li et al. 2009, 2011). Thus, A β -induced synaptic depression may result from an initial increase in synaptic activation of NMDARs by glutamate, followed by synaptic NMDAR desensitization, NMDAR/AMPA internalization, and activation of extrasynaptic NMDARs and mGluRs. A β -induced LTD-like processes may underlie A β -induced LTP deficits, as blocking LTD-related signaling cascades, such as mGluR or p38 MAPK, can prevent A β -dependent inhibition of LTP (Wang et al. 2004).

WHAT ARE THE RECEPTORS BY WHICH SOLUBLE OLIGOMERS PERTURB SYNAPTIC FUNCTION?

Although the many studies reviewed so far in this chapter have suggested some of the pathways through which elevated extracellular A β levels particularly in the form of soluble oligomers can alter synaptic transmission, precisely how soluble A β oligomers *initiate* effects on synaptic structure and function remains to be determined. Diverse lines of evidence suggest that extracellular oligomers can bind to pre- and postsynaptic elements on cultured neurons and in the AD cortex. Cellular and animal studies that have attempted to identify the molecular targets of the oligomers have yielded an array of candidates. A β has been reported to interact functionally—and sometimes also structurally—with several distinct types of plasma membrane-anchored receptors, including $\alpha 7$ nicotinic acetylcholine receptors, NMDA and AMPA receptors, insulin receptors, RAGE (the receptor for advanced glycation end-products), the prion protein, and the Ephrin-type B2 receptor (EphB2) (Yan et al. 1999; Lacor et al.

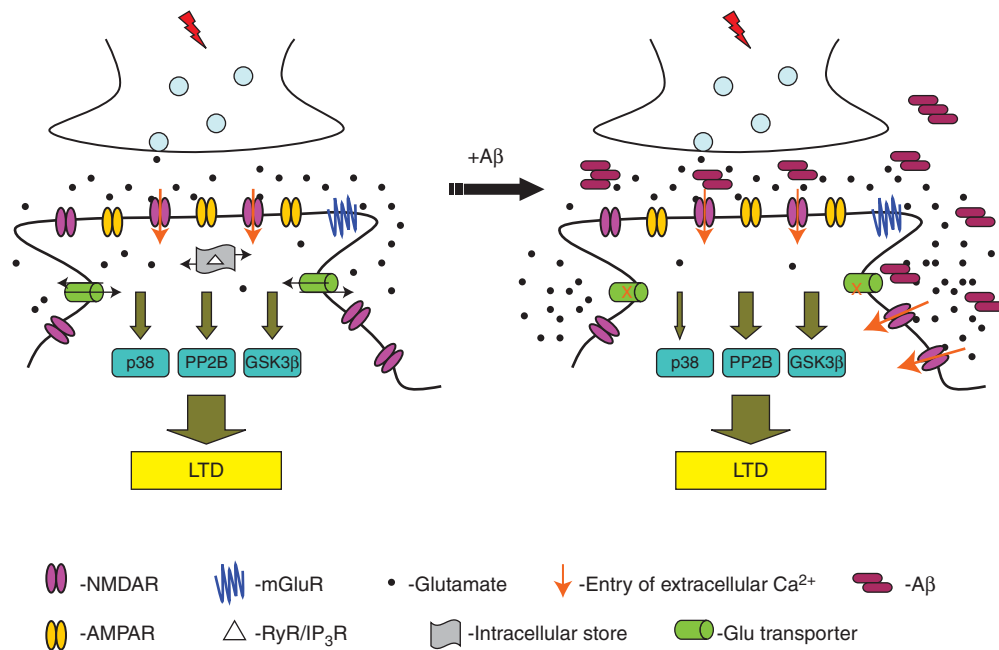


Figure 2. Schematic of the principal pathways implicated by this study in conventional LTD and in LTD facilitated by soluble Aβ oligomers (*left panel*). Conventional LTD requires NMDAR-mediated influx of extracellular calcium and liberation of intracellular calcium stores. This ultimately activates PP2B, GSK-3b, or p38 MAPK signaling pathways that induce LTD. (*Right panel*) Soluble Aβ oligomers lead to activation of more NMDAR, leading to extracellular calcium influx and activation of PP2B and GSK-3b pathways to facilitate LTD. Our data suggest that Aβ oligomers decrease glutamate uptake by neuronal transporters (red 'x's'), resulting in the enhanced activation of NMDARs and thus facilitation of LTD-inducing pathways.

2004; Verdier et al. 2004; Lacor et al. 2007; Simakova and Arispe 2007; Koffie et al. 2009; Lauren et al. 2009; Gimbel et al. 2010; Cisse et al. 2011a).

Several key questions should be considered in interpreting such studies. Have the investigators rigorously specified the form of Aβ that is binding to cultured neurons or brain sections and performed the binding studies under physiologically relevant Aβ concentrations and conditions? Many studies have used synthetic Aβ peptides of a single defined length (e.g., Aβ1–40) at potentially supraphysiological concentrations (e.g., 0.1–10 μM). Physiological concentrations of Aβ peptides in human brain, interstitial fluid and cerebrospinal fluid are in the low nanomolar range or below, although concentrations in the most pathobiologically relevant sites of the AD brain, for example,

within and around synaptic clefts, are unknown and might be higher. Are the receptor interactions the authors report occurring with just the monomer (a physiological peptide in mammals), just certain soluble oligomers (e.g., dimers, trimers, dodecamers), and/or just protofibrils? Have the authors used biochemical methods such as size exclusion chromatography under entirely nondenaturing conditions to isolate and specify a particular assembly form, and can they assume that this form has not changed during the experiment (e.g., by recovering it intact after the exposure to neurons)?

These questions are relevant to the issue of whether secreted, soluble Aβ monomers have cognate physiological receptors, analogous, for example, to tachykinin receptors for substance P. If they do (and none has yet been confirmed

unequivocally in multiple laboratories under physiological conditions), then are there entirely different receptors that bind soluble oligomers? One would think so, as the biochemical properties of oligomers are distinct from those of the secreted monomer; for example, they are folded differently. However, preferential binding to A β oligomers (as opposed to monomers) may not be required for a receptor to be a mediator of A β oligomer-induced neuronal dysfunction, because interactions of the receptor with these different A β species could elicit distinct signal transduction cascades. For example, dimerization of a particular receptor might be induced by A β oligomers but not monomers, despite comparable binding affinities. Ideally, interactions between A β oligomers and their receptor(s) would show classical ligand-receptor binding kinetics such as those of insulin and substance P with their cognate receptors. However, binding of A β oligomers to some putative receptors, for example, EphB2, triggers degradation of the receptor in the proteasome (Cisse et al. 2011a), which could result in more complex kinetics.

Because soluble oligomers (e.g., dimers, trimers, dodecamers) of A β 42 have exposed hydrophobic residues that allow them to bind additional monomers and they are thus highly sticky, it seems probable from a biophysical perspective that A β 42 interacts initially with other hydrophobic molecules, in particular membrane lipids, rather than relatively hydrophilic proteins like the ectodomains of the various candidate receptors mentioned above. Numerous studies using high levels of synthetic A β 40 or A β 42 indicate that such preparations can bind to membranes and perturb their structure, in some cases causing actual holes in the membrane that could conduct ions and thus induce cytotoxicity (Demuro et al. 2005; Lin et al. 2001). However, there is little evidence that such major membrane disruption occurs upon exposure of neurons to natural oligomers of secreted A β isolated from culture media or brain tissue and applied at nanomolar concentrations. More subtle but sustained (chronic) effects of A β oligomers on membrane lipids may well contribute to A β -induced neuronal

dysfunction (Sanchez-Mejia et al. 2008), which makes the further investigation of A β /lipid interactions an important objective.

Accordingly, we are in need of rigorous biochemical studies of fully purified natural monomers and oligomers isolated from AD brain tissue that are subsequently labeled, or else synthetic labeled oligomers with predetermined structures, allowing the performance of unbiased binding screens (e.g., using cross-linking) to identify which discrete surface molecules the monomers or the oligomers bind and what their binding kinetics are. Until such labor-intensive studies are performed by more than one laboratory, available data can only suggest that a particular receptor (e.g., the α 7-nicotinic ACh receptor) plays a required role in membrane engagement and anchoring of A β and/or its downstream biological effects, not that they necessarily represent the initial binding receptor. In addition, the pathophysiological role(s) of putative A β oligomer receptors should be validated rigorously in relation to clinically relevant functional outcome measures in different experimental models and by independent groups, using genetic and pharmacological manipulations as well as electrophysiological, radiological, and behavioral outcome measures. For example, in independent studies, PrPc ablation either did (Lauren et al. 2009; Gimbel et al. 2010; Barry et al. 2011) or did not (Kessels and Malinow 2009; Balducci et al. 2010; Calella et al. 2010; Cisse et al. 2011b) prevent A β -induced neuronal dysfunction, leaving the functional significance of an A β /PrPc interaction uncertain at this writing.

EXTRACELLULAR VERSUS INTRANEURONAL A β : EVIDENCE FOR AND AGAINST AN ATTACK BY A β FROM WITHIN THE NEURON

The classical histopathology of AD brains is characterized by large numbers of extracellular deposits of A β in the cortical neuropil and in blood vessel walls (see Serrano-Pozo et al. 2011). This principally extracellular location is consistent with the fact that A β arises from the intraluminal/extracellular cleavage of APP

by β -secretase followed by the intramembranous γ -secretase cleavages that release it from the membrane into the aqueous environment of the vesicle lumen or extracellular space (Haass et al. 1992, 2011; Shoji et al. 1992). Moreover, systemic amyloids are well known to occur in the extracellular space of various tissues, not intracellularly. The application to AD brain sections of monoclonal antibodies to epitopes that can only be on free A β (i.e., are not detectable in the A β sequence when it is within the APP molecule) generally reveals enormous amounts of extracellular A β -reactive material and little or no specific staining of cell bodies. However, some careful analyses have revealed the additional presence of intraneuronal A β immunoreactivity that appears to occur in the lumens of multivesicular bodies and some other types of intracellular vesicles (Takahashi et al. 2004, 2002; Gouras et al. 2005; Almeida et al. 2006). Such a locus is consistent with the cell biology of APP, as it has been shown in numerous studies that the proteolytic processing of APP to A β can occur in intracellular vesicles in the secretory and endosomal trafficking pathways. Uptake of A β 42 through the endosomal/lysosomal pathway has been reported to cause lysosomal leakage (Yang et al. 1998), which could provide A β with access to the cytosol, although the normal occurrence of cytosolic A β has not been widely confirmed. The possible association of A β with mitochondria (Chen and Yan 2007) also suggests that A β can exist in these compartments. It is important to reiterate that intracellular A β can only be established using end-specific A β antibodies that are incapable of reacting with A β sequences within APP and its proteolytic products present abundantly inside neurons, as has recently been emphasized (Winton et al. 2011).

The interpretation of the intravesicular A β -reactive peptides reported in neurons is not entirely clear. These peptides could represent small amounts of A β produced by normal APP processing that is destined for secretion, or they may be in the process of being targeted for proteolytic degradation in the late endosomal/lysosomal system (see Ihara et al. 2011). It is also possible that they could represent

previously secreted A β monomers and/or oligomers that have been taken back up into cells. In this regard, it is of interest that apparent dimers of A β have been detected by immunoprecipitation/western blotting in vesicles isolated from APP-expressing cells, including neurons (Walsh et al. 2000). The highly compact space of a vesicle lumen could afford the molecular crowding that A β monomers may need to enhance the chances of oligomer formation, compared with the relatively dilute state of the extracellular/interstitial fluid. Another topic for consideration is whether synaptic dysfunction and neurotoxicity arise principally from intracellular A β or from the far more abundant extracellular stores of monomers and oligomers found in AD brains or from both. Clearly, the extracellular application of biochemically isolated natural oligomers of A β at physiological concentrations has been shown to induce extensive neuronal changes, including altered synaptic plasticity and synapse form (Klyubin et al. 2005; Shankar et al. 2007, 2008; Li et al. 2009), abnormal tau phosphorylation progressing to neuritic dystrophy (Jin et al. 2011), and interference with memory (Cleary et al. 2005; Lesne et al. 2006; Shankar et al. 2008). One does not yet know whether a solely intraneuronal accumulation of such soluble oligomers is sufficient to induce these various AD-like phenotypes.

DISRUPTION OF COGNITIVE FUNCTIONS: FROM SYNAPSES TO NEURAL NETWORKS

The dynamic complexity of A β assembly forms is easily matched, if not outdone, by the complexity of the neural networks on which they act. Distributed networks such as the so-called “default network” comprise different brain regions, which, in turn, contain multiple interconnected circuits that are made up of distinct cell types and myriad synaptic contacts. A key unresolved question in the AD field is whether A β assemblies affect different neurons and synapses differentially. Answering this question is critical if one wants to predict the effects of A β on the output of neuronal circuits and the activity of networks (Palop et al. 2006; Palop

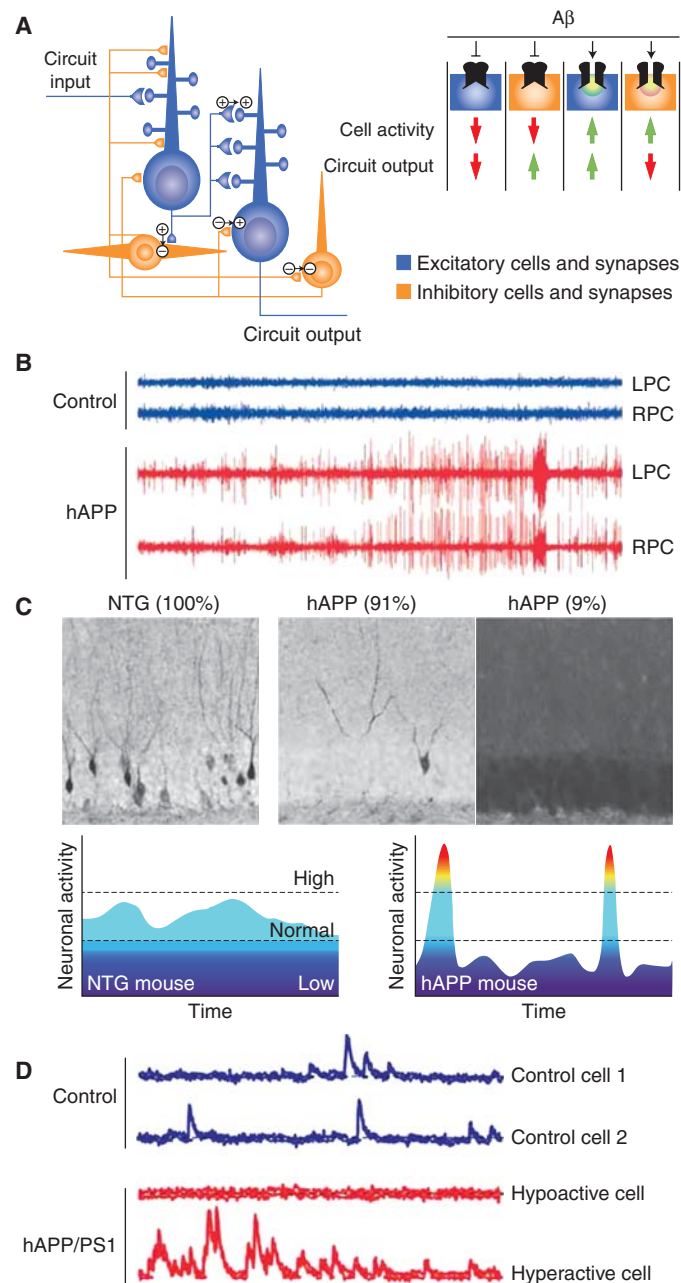


Figure 3. Pathologically elevated A β elicits abnormal patterns of neuronal activity in circuits and in wider networks in Alzheimer disease–related mouse models. (A) Neuronal circuits are formed by synaptic interactions between excitatory and inhibitory cells. A β might differentially affect excitatory (+) and inhibitory (−) synapses and cells, producing complex imbalances in circuit and network activity. (B) At the network level, high levels of A β increase network synchrony and elicit epileptiform activity, as illustrated here in EEG recordings from the left and right parietal cortex (LPC and RPC, respectively) of nontransgenic (NTG) controls (blue) and hAPP transgenic mice from line J20 (red). (C) hAPP mice show fluctuations in the neuronal expression of synaptic activity–dependent genes, suggesting network instability. (See facing page for legend.)

and Mucke 2010). For example, if A β impaired the synaptic function of inhibitory interneurons more than that of excitatory principal cells, it would be likely to cause disinhibition and overexcitation, rather than suppression, at the network level (Fig. 3). A similar effect would result if A β suppressed excitatory \rightarrow inhibitory synapses more than excitatory \rightarrow excitatory synapses.

Experimental evidence suggests that some GABAergic neurons may indeed be especially vulnerable to A β , which helps explain apparent discrepancies between results obtained in analyses of specific synapses versus circuits and networks. Early electrophysiological studies focused on two specific glutamatergic synapses in the hippocampus, the CA3 \rightarrow Schaffer collateral \rightarrow CA1 pyramidal cell synapse and the entorhinal cortex \rightarrow perforant path \rightarrow dentate gyrus granule cell synapse. At these synapses, A β has been reported in numerous studies to suppress transmission strength and/or long- and short-term plasticity (see Palop and Mucke 2010 for review). Based on just these two circuits, it might be expected that A β primarily suppresses network excitability, but this is not what actually happens in vivo.

Among the first clues suggesting that A β can elicit aberrant excitatory activity at the network level was the identification of anatomical and biochemical alterations in the dentate gyrus of hAPP mice that are typically seen in rodent models of epilepsy or other states of neuronal overexcitation. These alterations include reductions in calbindin and increases in neuropeptide Y (NPY; Palop et al. 2003; Palop et al. 2007). Video electroencephalogram (EEG) telemetry recordings in freely behaving hAPP mice have detected widespread cortical and hippocampal

epileptiform activity (Palop et al. 2007; Minkeviciene et al. 2009; Vogt et al. 2009; Roberson et al. 2011). Some of these EEG studies also documented intermittent, nonconvulsive seizures that were difficult or impossible to detect by visual observation. Although some lines of hAPP mice have frank convulsive seizures, such events appear to be rare in most hAPP mice. Convulsive seizures are also rare in sporadic AD, although this condition is clearly associated with an increased incidence of epilepsy (reviewed in Palop and Mucke 2009). Interestingly, clinically apparent seizures are much more common in cases with early-onset AD, particularly autosomal dominant pedigrees and AD associated with Down syndrome, suggesting a potentially causal role of high A β levels and aggressive cytopathology. The incidence of nonconvulsive (subclinical) seizure activity in familial and sporadic AD is unknown. Studies have recently been launched in multiple centers to address this intriguing issue.

Additional studies are also needed to elucidate the precise mechanisms by which A β elicits aberrant excitatory network activity. The possibilities include direct proexcitatory effects on principal glutamatergic neurons and impairments of inhibitory interneurons that therefore indirectly disinhibit the network (Palop et al. 2007; Busche et al. 2008; Palop and Mucke 2010). Acutely, exposure to synthetic or natural A β assemblies can increase neuronal activity in cell culture and cortical slices (Sanchez-Mejia et al. 2008; Supnet and Bezprozvanny 2010). The underlying mechanisms may involve increases in $[Ca^{2+}]_i$, activation of group IVA phospholipase A₂ (GIVA-PLA₂), increased release of arachidonic acid, and/or transient increases in surface levels of glutamate

Figure 3. (Continued) *Top:* Compared with NTG controls (*left*), hAPP-J20 mice show abnormally low (*middle*) or high (*right*) Arc expression in granule cells of the dentate gyrus (adapted, with permission, from Palop et al. 2005, 2007). Percentages indicate the proportion of mice showing the different patterns of Arc expression. Such marked increases in Arc expression are typically caused by seizure activity. *Bottom:* Interpretive diagram. Marked fluctuations in neuronal activity may directly impair cognition by reducing the time the network spends in activity patterns that promote normal cognitive functions. (*D*) In cortical circuits of mice monitored in vivo by calcium imaging, most neurons in NTG controls (blue traces) have an intermediate level of activity, whereas many neurons in hAPP/PS1 transgenic mice with high A β levels (red traces) are either hypoactive (*top*) or hyperactive (*bottom*). (Adapted, with permission, from Palop and Mucke 2010.)

receptors. Chronically, A β appears to interfere with neuronal glutamate transporters, resulting in increased levels of glutamate in and around the synaptic cleft, desensitization of glutamate receptors and engagement of LTD-related signaling pathways (Hsieh et al. 2006; Li et al. 2009; Wei et al. 2010). Pathogenic glial loops resulting in the production of excitotoxins may contribute as well. A β also increases met-enkephalin levels in the hippocampus and entorhinal cortex, which could suppress the activity of inhibitory interneurons via stimulation of μ -opioid receptors. Indeed, pharmacological blockade of these receptors improved the performance of hAPP mice in the Morris water maze (Meilandt et al. 2008).

Overexcitation or hypersynchrony of neural networks triggers a multitude of compensatory responses, including extensive remodeling of neuronal circuits. This leads to a complex combination of decreased (probably primary) and increased (probably secondary) inhibitory pathways. For example, whereas hAPP mice show evidence for impaired function of GABAergic interneurons (Busche et al. 2008; Roberson et al. 2011), the outer molecular layers of their dentate gyri have extensive sprouting of GABAergic terminals, and their granule cells receive an increased number of inhibitory inputs (Palop et al. 2007). In addition, their mossy fiber collaterals contact basket cells, which would be expected to result in feed-forward inhibition of the granule cells from which the mossy fibers emanate. These alterations are consistent with the idea that the dentate gyrus, which epileptologists regard as the “gate” to the hippocampus, can activate mechanisms to block A β -induced aberrant excitatory activity. Much of this excess activity probably originates in cortical areas (Harris et al. 2010).

It is likely that, in AD and mouse models thereof, compensatory inhibitory mechanisms manage to delay and diminish excitotoxic processes that ultimately cause loss of synapses and neurons. However, these mechanisms may simultaneously constrain the agility of excitatory processes required for normal learning and memory. In addition, they probably contribute to a “yin and yang” between too much and too

little neuronal activity, diminishing the amount of time networks spend in a physiological range of activity that is conducive to normal cognitive functions (Fig. 3C). Direct evidence for such fluctuations comes from studies monitoring neuronal expression of the activity-related gene product Arc in dentate granule cells or calcium fluxes in neocortical neurons of live hAPP mice (Palop et al. 2007; Busche et al. 2008).

THERAPEUTIC IMPLICATIONS OF THE CONCEPT THAT A β -MEDIATED NEUROTOXICITY OCCURS PRINCIPALLY AT THE LEVEL OF SYNAPTIC NETWORKS

What are the therapeutic implications of the complex synaptic and network alterations reviewed in this chapter? First, AD is a slowly progressive and highly dynamic process, with different mechanisms probably predominating at different stages of the disease. Supporting this notion, recent studies show that detrimental effects of A β on adult-born granule cells can be prevented by inhibiting GABA_A receptors during early stages of their development or by enhancing glutamatergic signaling during later stages of maturation (Sun et al. 2009). Second, if aberrant increases in network excitability or synchronization are indeed early/proximal events in the A β -triggered pathogenic cascade, identifying ways to block this process becomes a critical therapeutic objective. The effect of antiepileptic drugs has not yet been rigorously evaluated in patients with early AD, and the optimal drug to block A β -induced aberrant excitatory neuronal activity in experimental models has yet to be identified. It will probably have to target the specific mechanisms by which A β elicits aberrant excitatory neuronal activity, which also remain to be pinpointed.

A pragmatic way forward to deal with the complex cellular and network alterations that occur during AD is to lower the levels of A β itself by inhibiting its production or enhancing its removal (see Schenk et al. 2011). Several such strategies are currently being assessed in human trials. At this writing, it remains unsettled whether such strategies will be efficacious and

safe (Golde et al. 2011; Selkoe 2011). For some of them, it is still uncertain whether they actually lower the levels of those A β assemblies that have the greatest impact on neuronal form and function. It therefore makes sense to complement these approaches with strategies that might make the brain more resistant to A β by targeting copathogenic factors or downstream mechanisms.

Examples of the latter strategies include reductions in the levels of the microtubule-associated protein tau (Roberson et al. 2007, 2011; Ittner et al. 2010; Jin et al. 2011; Morris et al. 2011) or of GIVA-PLA₂, (Sanchez-Mejia et al. 2008) or replacement of apoE4 function with apoE3-like function (Raber et al. 2000; Buttini et al. 2002; Mahley et al. 2006). Although the precise mechanisms by which these and similar interventions prevent A β -induced cognitive impairments without reducing A β levels remain to be determined, they may share a general effect of making the brain more resistant to aberrant excitatory synaptic activity. For example, even partial (50%) reduction of endogenous wild-type murine tau prevented synaptic and behavioral deficits in hAPP-J20 mice as well as evidence of neuronal overexcitation (Roberson et al. 2007; Morris et al. 2011; Roberson et al. 2011). Surprisingly, it did so without affecting A β levels, plaque formation or neuritic dystrophy. Similarly, knock-down of tau in cultured neurons made them markedly resistant to the cytoskeletal disruption and neuritic dystrophy induced by natural oligomers of A β isolated from AD cortex (Jin et al. 2011). Hippocampal slices from tau knockout mice were resistant to the LTP inhibition caused by synthetic A β peptide (Shipton et al. 2011). Tau reduction has also been shown to make mice with or without hAPP/A β overexpression more resistant to chemically induced seizures (Roberson et al. 2007; Ittner et al. 2010), suggesting a previously unrecognized role of tau in the regulation of neuronal activity. These various findings raise the intriguing possibility that nonaggregated wild-type tau fulfills a normal neuronal function that is required for A β and other excitotoxins to elicit aberrant excitatory activity.

Although the relative amounts of specific isoforms of tau and its exact amino acid sequence differ in mice and humans, the longest tau isoforms expressed in human and mouse brain are 88% identical and 92% similar. Proteins that are this highly conserved in amino acid sequence are likely to have conserved functions. Therefore, investigating the functions of mouse tau in transgenic models should provide clues regarding the roles of human tau in health and disease. This is particularly so because the enabling role of endogenous tau in A β -induced neuronal dysfunction probably does not depend on direct interactions between A β and tau, which are localized to separate compartments of the neuron. Instead, it may depend on permissive activities of tau, such as facilitation of neuronal excitability (Ittner et al. 2010), that are likely conserved in mice and humans. Such hypothetical tau functions could play a critical role in the pathogenesis of dementia and are not inconsistent with evidence that pathogenic tau aggregates cause neurodegeneration in AD and other tauopathies (Hoover et al. 2010; Zempel et al. 2010).

Intuitively, it makes sense that loss of neurons is a principal basis for cognitive decline in AD and other neurodegenerative dementias. However, several observations suggest that one should not view it as the *sine qua non* of functional decline, particularly early on in the syndrome. For example, the brain can compensate quite well for major losses of neurons, especially when these losses occur over prolonged periods of time. A striking example is a patient with longstanding communicating hydrocephalus who has only a rim of cortical ribbon left but functions quite well despite remarkably abnormal brain scans (Lewin 1980). Importantly, in APP transgenic mice, A β accumulation elicits severe synaptic impairments and unequivocal deficits in learning and memory without causing major neuronal loss, although neurites do degenerate. In tau transgenic mice, cognitive deficits are associated with neuronal loss, but these deficits can be reversed despite the persistence of the neuronal loss (SantaCruz et al. 2005).

Taken together, available data reviewed here and elsewhere raise the possibility that a

significant proportion of the profound cognitive and behavioral deficits in AD patients are due to the dysfunction of synapses and neural networks (Selkoe 2002; Palop and Mucke 2010). This concept has far-reaching therapeutic implications. While the replacement and proper integration of whole neurons remains a very major challenge, the regeneration of neurites, the re-establishment of synaptic contacts and an improvement of network function appear within somewhat closer experimental reach. Fostering such restorative processes while also trying to diminish or block factors that fuel the progression of AD, such as A β and tau accumulation, should slow and ultimately even prevent cognitive dysfunction in this dauntingly complex syndrome.

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