To Think or Not to Think: Synaptic Activity and Aβ Release

Accumulation of β-amyloid protein (Aβ) in the extracellular space of the brain has been hypothesized to be a culprit in the pathogenesis of Alzheimer's disease. In this issue of *Neuron*, Cirrito et al. describe a series of experiments demonstrating that extracellular Aβ levels are directly modulated by neuronal and synaptic activity.

Alzheimer's disease, the most common form of dementia in the elderly, is a progressive neurodegenerative disorder characterized by a number of pathological features including extracellular deposits known as senile plaques. Beyond postmortem characterization, however, the pathogenesis of Alzheimer's disease is still unclear in many respects. One of the most likely culprits underlying Alzheimer's disease is the β-amyloid protein (Aβ), acting through the amyloid cascade hypothesis. For example, fibrillar Aβ is the primary component of the plaques that ravage the brains of Alzheimer's disease patients. Additionally, Alzheimer's pathology invariably develops in those carrying an extra copy of the amyloid precursor protein (APP) gene (as is present in those with trisomy 21). Finally, a number of familial genetic mutations have been found that result in early-onset Alzheimer's disease. In these families, alterations in either APP or in the proteins known to cleave APP (presenilins, for example) have in common the function of increasing the production of Aβ or the more amyloidogenic and putatively more toxic $A\beta_{42}$ species.

While the precise mechanisms by which Aβ exerts its pathogenic effects are not clear, a number of key principles have emerged. For example, much research has focused on the idea that Aβ may exert deleterious effects on synaptic function—effects that occur before outright cell death. Changes in the density of synaptophysin, as a marker of synaptic number, are better correlated with disease progression than plaque load or cell death (Terry et al., 1991). In transgenic mouse models of amyloid deposition, numerous groups have shown deficits in electrophysiological measures of synaptic communication or plasticity (for example, Hsia et al., 1999), which can be reversed when amyloid load is lowered by passive immunization. Lastly, and most recently, the importance of the aggregation state of Aβ has become apparent; for example, soluble Aβ oligomers appear to be a necessary component in the Aβ-induced inhibition of long-term potentiation (Klyubin et al., 2005).

Evidence suggests that the concentration of Aβ in the extracellular space plays an important role in the aggregation state and therefore the toxicity of the Aβ protein (Meyer-Luehmann et al., 2003). Thus, given the apparent neurotoxic and synaptotoxic effects of Aβ, factors that influence the production or clearance of Aβ, which in turn contribute to the overall levels of extracellular Aβ in brain, are of obvious importance. In this issue of *Neuron*, Cirrito et al. (2005), in a technological tour de force, examine a promising angle of research in this direction—the idea that neuronal activity plays a causal role in determining the levels of extracellular Aβ. A role for neuronal activity in Aβ release has previously been demonstrated (Kamenetz et al., 2003); however, adequate gauging of both the anatomical and temporal components involved in neuronal activity-induced Aβ accumulation has not previously been attempted. With the use of a combination of in vivo microdialysis, electrophysiological recording, and in vivo pharmacology, Cirrito et al. were able to address the dynamic changes of extracellular Aβ levels in response to neuronal stimulation in awake, behaving mice as well as in hippocampal slice preparations.

In their first experiment, Cirrito et al. stimulated the perforant pathway (the major afferent axon route leading from the entorhinal cortex into the hippocampus) while recording electrophysiological activity in the hippocampus and, at the same time, sampling interstitial fluid (ISF) via microdialysis. Continuous perforant pathway stimulation, which caused epileptiform activity within the hippocampus, caused ISF Aβ to increase by 30% within 1 hr, demonstrating a direct relationship between neuronal activity and ISF Aβ. Conversely, infusion of tetrodotoxin (TTX), which blocks sodium channels and causes a cessation of neuronal activity, significantly decreased basal electrophysiological activity and dropped Aβ levels by 40%. Importantly, this effect was reversible, signaling a direct causal relationship between neuronal activity and ISF Aβ levels. Furthermore, the drop in Aβ accumulation was shown to be a result of synaptic release, rather than broad neuronal activity. Infusion of tetanus toxin, which inhibits synaptic vesicle release, had only a small effect on EEG amplitude, but Aβ levels decreased by 70%—an even greater decline than following TTX treatment.

While these findings and others like them appear to confirm that neuronal, and, in particular, synaptic activity does increase the concentration of extracellular Aβ, the question remains as to the mechanism by which Aβ is increasing. Two possibilities appear likely: first, the half-life of extracellular Aβ may be extended; or second, there could be an effect on intracellular APP processing. Using a γ-secretase inhibitor (blocking new generation of Aβ) in TTX and vehicle-treated mice, Cirrito et al. showed that Aβ half-life was unaltered by depression of neuronal activity. Additionally, expression of the Aβ-degrading enzyme neprilysin was unchanged in TTX-treated mice. Support for the latter possibility (APP processing changes) was provided by Kamenetz et al. (2003). In the Cirrito et al. study, though, TTX-induced reduction in extracellular Aβ did not result in changes in β-CTF levels. There was a significant 13% increase in α-CTF level; however, this increase is unlikely to explain the much larger change in Aβ levels.

In order to further investigate this discrepancy and elucidate the mechanism underlying neuronal activity-induced Aβ alterations, Cirrito et al. treated hippocampal slices with several compounds: α-latrotoxin, which

induces synaptic vesicle release in the absence of presynaptic depolarization, and/or a neuronal activity inhibitor cocktail containing both presynaptic and postsynaptic activity inhibitors. These experiments confirmed that synaptic release, even in the absence of neuronal activity, increases extracellular Aβ levels. These changes were again independent of alterations in CTF levels, however, indicating that, at least in this time frame, extracellular Aβ modulation appears to be directly related to synaptic vesicle release rather than via changes in Aβ clearance or APP processing.

The results presented in the Cirrito et al. study have confirmed the general tenet espoused by Kamenetz et al. (2003) and several other groups—that neuronal or synaptic activity can directly influence Aβ levels. With this finding well supported, the more relevant question appears to be related to the mechanism proposed to underlie this effect. In the Kamenetz et al. study, increasing neuronal activation via a 36 hr picrotoxin treatment caused a significant increase in secreted Aβ that was accompanied by a 3-fold increase in β-CTF expression. Conversely, TTX-treated neurons showed a 50% decrease in Aβ and an associated drop in β-CTF expression. The results thus suggested that the level of BACE cleavage could be controlled by neuronal activity. This change could be due to an alteration in BACE activity itself. Alternatively, if one assumes that BACE cleavage can take place within axon terminals, increased membrane recycling following neuronal activity would lead to a greater amount of APP available for processing.

Interestingly, the results of the present study suggest that rather than requiring changes in APP processing, synaptic activity-dependent Aβ alterations are accomplished via a mechanism related specifically to vesicle release. Specifically, β-CTF levels were unchanged following either TTX treatment (reducing neuronal activity) or α-latrotoxin treatment (inducing synaptic release), suggesting that altered BACE-dependent activity is not required or responsible for the increased extracellular Aβ following synaptic activity. The apparent discrepancy between the present study and the Kamenetz et al. study may be explained by differences in the treatment regimen. Whereas Kamenetz et al. treated brain slices with picrotoxin or TTX for 36 hr, inducing changes in β-CTF expression, in the present study treatments were shorter and more anatomically localized in the in vivo setting (16 hr via hippocampal infusion) and much shorter in hippocampal slices (2 hr). The effect of neuronal activity on BACE cleavage of APP may thus be dependent on time or area of exposure.

In the absence of BACE cleavage alterations and apparently new Aβ generation, where is all of this Aβ coming from? Given that Aβ appears to be released synaptically (both neuronal activity and induced vesicular release cause increases in Aβ), one distinct possibility is that axon terminals may contain a readily releasable pool of Aβ, much like neurotransmitter residing within synaptic vesicles at presynaptic terminals. What compartment, presumably vesicular in nature, Aβ is residing in is unclear. To date, neither APP nor Aβ is known to be sorted into synaptic vesicles (Marquez-Sterling et al., 1997). Thus, these vesicles are apparently distinct from neurotransmitter-containing synaptic vesicles yet respond in an exocytic manner secondary to synaptic stimulation. If this hypothesis is correct, it is also interesting to speculate whether synaptic Aβ release would undergo "fatigue" in the same way that neurotransmitter release can be hindered by overstimulation. However, whether one can measure release of Aβ in the time frame necessary to answer this question is an understandably dubious endeavor.

A neuronal origin of Aβ is generally accepted. This is best seen by the dramatic loss of amyloid deposits in brain following transection of corresponding axonal tracts (Lazarov et al., 2002). However, it remains unclear whether Aβ is formed upon leaving the cell body, enroute in axons, or at the presynaptic terminals. Thus, the synaptic release of Aβ from an apparently readily releasable pool has potential implications on the site of Aβ generation within neurons. On the surface then, this notion is consistent with the controversial model proposed by the Goldstein laboratory, wherein Aβ may be produced during axonal transport of APP (Kamal et al., 2001, Lazarov et al., 2005). One can envision Aβ formed along the axon or originating from the cell body accumulating at the presynaptic terminal until released exocytically. On the other hand, because BACE and presenilins are also axonally transported, it is possible that APP is proteolytically processed into Aβ within a vesicular compartment at synaptic terminals that await a stimulus for exocytic release. Perhaps the interesting observations in this study will provide the necessary impetus to resolve the precise sites of Aβ generation in neurons.

In addition to the cellular issues of APP processing and Aβ release, the idea that Aβ levels are correlated to synaptic activity also has interesting implications at higher levels of examination. For some time, several lines of inquiry have seemingly pointed to synaptic activity as a "good" thing in the aging process. For example, educational level has been negatively correlated with Alzheimer's disease likelihood. Similarly, aging individuals have been encouraged to "keep their mind sharp" by doing crossword puzzles, word jumbles, and the like. Seemingly at odds with these ideas, recent evidence has shown that the brain regions with the highest basal activity in terms of metabolic rate—the so-called "daydreaming" areas—are also the most prominently affected by Aβ plaques (Buckner et al., 2005). These results offer an explanation as to why certain brain regions are consistently affected in Alzheimer's patients, while other regions are not (assuming that basal metabolic increases are resulting in increased extracellular Aβ accumulation). So, is synaptic activity a good thing or a bad thing? The question may hinge on whether one is talking about acute activity (such as in the present study) or long-term activity. One possibility that might explain the apparent paradox is that mental activity (such as working crossword puzzles…, etc.) may serve to activate memory circuits in the short term, but could perhaps decrease basal activity in these same circuits in the long term—thus providing a protective effect against the activity-driven release of Aβ.

Perhaps the most important question remaining unanswered is: does this transient pool of synaptically released Aβ modulated by brain activity play a role in the pathogenesis of Alzheimer's disease? While technologically remarkable and theoretically intriguing, the present study does achieve results in a nonphysiological

manner. For example, Aβ enhancement was achieved by induction of epileptic seizure activity in the hippocampus. One might assume, therefore, that patients with epilepsy would be at higher risk of Aβ accumulation; however, that has proven not to be the case. Instead, this model of transient increase in Aβ release may be closer to that seen following traumatic brain injury, a known risk factor for AD, where there is a brief rise in Aβ levels detected in brain and cerebrospinal fluid (Chen et al., 2004). In turn, this is correlated to formation of diffuse amyloid deposits in the brain. Notably, while the physiological relevance of synaptically released Aβ is not completely understood at present, the fact that pharmacological inhibition of synaptic vesicle release can decrease extracellular Aβ by nearly 90% is certainly strong evidence that this pool of Aβ is a potentially significant source of Aβ. Future studies will undoubtedly shed more light on its functional importance.

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Dystonia and the Nuclear Envelope

Mutations in torsinA cause dominantly inherited early-onset torsion dystonia in humans. In this issue of *Neuron*, Goodchild et al. show that torsinA knockout and knockin mice have similar phenotypes, which suggests that the mutant torsinA allele causes disease because it has decreased function. The experiments also highlight the possible role of nuclear envelope dynamics in maintaining normal neuronal function.

Many neurological diseases have variants that are caused by gene defects. The relationships between Mendelian forms of disease and their sporadic counterparts can be debated, but there is a chance of understanding the basic pathways leading to neuronal dysfunction. However, the usefulness of this information is predicated on understanding how the mutation "works" in a genetic sense. For example, a recessive mutation is usually associated with loss of function, which could be tested experimentally if one has an assay for the protein activity. Dominant mutations may work in any number of ways, by increasing the activity of the protein, introducing a new toxic property to the protein such as protein misfolding (gain of novel function), or acting to reduce the activity of the endogenous protein (dominant negative). These distinctions are critical for designing the right experiment to test hypotheses about how diseases are caused and misapplication really causes problems in interpretation. Such information may also change how we design therapeutic strategies and hence is of significant interest.

The genetics of the human dystonias are complicated as there are at least 15 extant loci with different phenotypic presentations (Klein, 2005). The first identified gene was named torsinA by Ozelius and colleagues and is associated with early-onset torsion dystonia (Ozelius et al., 1997). All torsinA-related dystonia cases found so far are due to the deletion of one of a pair of glutamate residues (E302/303) toward the C terminus of the encoded protein. TorsinA is part of the large AAA+ family of ATPases and the glutamate deletion is near to the ATP binding region. An interesting aspect of the genetics of torsinA dystonia is that there is greatly reduced penetrance. About one third of patients who carry the causal ΔE302/303 mutation go on to develop disease, the rest remaining asyptomatic. It is also worth noting that the there appears to be a time-dependent window for susceptibility. Generally, mutation carriers who are asymptomatic in their early 20s remain so throughout life, although there may be exceptions (Bressman et al., 2000). This implies that there is a critical timing for the expression of symptoms, also implying that dystonia is a developmental disease.

The interpretation of this human genetic evidence is not simple because there are multiple reasonable explanations for both the low penetrance dominant disease and the developmental effects. The study by Goodchild et al. is important because it addresses this problem in a careful but direct manner. The question posed by the experiments is: how closely does the ΔE302/303 mutation resemble a loss of function allele? The authors address this by comparing the phenotypes of mice with a knockout of torsinA or a knockin mutation in which one of the pair of glutamate residues is removed. If the two mice are similar in phenotype, this would argue quite strongly and in a relevant in vivo context, that the mutation causes a loss of function.