

Discussion

Axonal transport hypothesis moves on to implicate presenilin¹: Alzheimer research forum live discussion

Jorge Busciglio and Scott Brady led this live discussion, an update of last year's initial Live Discussion of axonal transport as an underlying factor in neurodegeneration.

<http://www.al2forum.org/nes/for/journal/busciglio/default.asp>

Participants: Akihiko Takashima, (Institute of Physical and Chemical Sciences (RIKEN), Japan); Peter Klein, (University of Pennsylvania); Leo Kim, (University of Pennsylvania); Frank LaFerla, (University of California, Irvine); Changiz Geula, (Beth Israel Deaconess Medical Center, Boston Massachusetts); Jorge Busciglio, (University of California, Irvine); Scott Brady, (University of Illinois at Chicago College of Medicine); Martha Stokely, (University of Florida Gainesville); Nikolaos Tezapsidis, (Columbia University); Gunnar Gouras, (Cornell University); Gabrielle Strobel, (Alzheimer Research Forum); Keith Crutcher, (University of Cincinnati, Ohio); Diego Forero, (National University of Colombia, Bogota).

Background text by Jorge Busciglio and Scott Brady

Neuronal cells are highly sensitive to transport defects because of their highly polarized morphology and large number of specialized microdomains. For their survival and proper function, neurons depend on the efficient delivery of proteins from the cell body to neuritic processes. Axons in particular are highly susceptible to transport deficiencies because they lack the elements necessary for protein synthesis. In this context, we have suggested that defects in protein transport play a critical role in Alzheimer's disease (AD) and other neurodegenerative conditions [1]. Experimental evidence indicates that kinase and phosphatase activities are key regulators of fast axonal transport. Two major serine-threonine protein kinases, glycogen synthase kinase-3 β (GSK-3 β) and cyclin-dependent kinase 5 (CDK5), have been implicated as major kinases responsible for both normal and pathological phosphorylation of tau

protein in AD. Moreover, GSK-3 β , which is highly homologous to GSK-3 β , has been recently implicated in the modulation of A β production [2]. Both CDK5 and GSK-3 β have also been shown to regulate kinesin-driven motility. Specifically, GSK-3 β phosphorylates kinesin light chains *in vivo* and causes the release of kinesin from membrane-bound organelles (MBOs), leading to a reduction in kinesin-I-driven motility [3]. Given the essential role of axonal transport in neuronal function, a misregulation of transport induced by an imbalance in specific kinase/phosphatase activities within neurons may represent an early and critical step of neuronal pathology.

Presenilin Mutations: They Do More Than Increase A β

Significant evidence indicates that presenilin-1 (PS1) is essential for gamma-secretase activity. At the same time, there is also considerable evidence to suggest that PS1 has additional physiological functions, including control of calcium homeostasis, cell-cycle regulation, neurite outgrowth, apoptosis, membrane traf-

¹Note: The transcript has been edited for clarity and accuracy.

ficking, and synaptic plasticity. In particular, PS1 has been implicated in regulating intracellular trafficking, maturation, and delivery to the cell surface of selected transmembrane proteins. Such effects of PS1 have been shown for the membrane proteins A β PP, TrkB and ICAM-5/telencephalin [4–6]. One way in which PS1 might modulate intracellular protein trafficking is by regulating kinesin-based motility.

To determine whether PS1 alters kinesin-based protein transport, we utilized presenilin-1 knockout (PS1 $^{-/-}$) and mutant human PS1 knock-in M146V (KIM146V) mice and cultured their cells. We show that PS1 and GSK-3 β coimmunoprecipitate and colocalize in specific neuronal compartments, particularly growth cones [7]. Both FAD mutations in PS1, or the absence of PS1, increased relative levels of GSK-3 β activity. One possibility is that both PS1 and GSK-3 β may be components of a trafficking regulatory complex at specific subcellular locations that is misregulated by the absence of PS1 or by the presence of PS1 mutations. Concomitant with increased GSK-3 β activity, PS1 deletion or PS1 mutations increased relative levels of kinesin light chain phosphorylation, and markedly reduced the amount of kinesin bound to MBOs. Consistent with a deficit in kinesin-mediated fast axonal transport, densities of synaptophysin and syntaxin-I containing vesicles and mitochondria were reduced in neuritic processes, but not cell bodies, of KIM146V hippocampal neurons. Similarly, we found reduced levels of PS1, A β PP, and synaptophysin in sciatic nerves of KIM146V mice. These results suggest that PS1 modulates GSK-3 β activity and normally affects the release of kinesin from MBOs at sites of vesicle delivery and membrane insertion.

In summary, we propose a model whereby mutations in PS1 compromise neuronal function by misregulating GSK3 activity, which would cause premature release of kinesin cargoes and impaired delivery of MBOs such as mitochondria to appropriate neuronal compartments. This ARF discussion will focus on the molecular mechanisms that may lead to disturbances of axonal transport and neurodegeneration in AD, including PS1 mutations, altered GSK3 kinase activity, tau hyperphosphorylation, and A β production.

Gabrielle Strobel: Hi, and welcome everyone. I am the editor of Alzforum and pleased to moderate today. Perhaps Jorge could start things off with a brief restatement of his hypothesis

Jorge Busciglio: We are studying the role of presenilin 1 (PS1) in axonal transport due to its interaction and

regulation of glycogen synthetase kinase 3 β (GSK-3 β) activity, which modulates kinesin phosphorylation and anterograde axonal transport. PS1 mutations appear to alter GSK-3 β and kinesin phosphorylation states.

Changiz Geula: Jorge and Scott, is there evidence that GSK-3 β directly phosphorylates kinesin *in vivo*?

Scott Brady: Yes. We published an extensive study in EMBO J last year [3]. GSK-3 specifically phosphorylates kinesin light chains, which then recruit the chaperone HSC70 to the vesicle and lead to removal of kinesin from the vesicle.

Jorge Busciglio: GSK-3 β phosphorylates kinesin light chain.

Gabrielle Strobel: What causes the proposed imbalance of kinase/phosphatase activity in aging humans? I understand you propose that familial Alzheimer disease (FAD) mutations disturb GSK-3 activity, but how about the majority of nonfamilial late onset AD cases?

Jorge Busciglio: Excellent question, Gabrielle, but a difficult one to answer.

Akihiko Takashima: Gabrielle, we have some results on GSK-3 β protein being upregulated during aging [8]. Protein phosphatase 2A (PP2A) mRNA levels are also downregulated during aging and in AD.

Gabrielle Strobel: Akihiko, what is PP2A? Does it regulate GSK-3 levels?

Akihiko Takashima: Gabrielle, PP2A regulates GSK-3 β activity by dephosphorylating serine 9.

Scott Brady: Akihiko, other phosphatases can also dephosphorylate GSK-3. We have evidence of a role for protein phosphatase 1 (PP1).

Akihiko Takashima: Scott, there was a nice article on interaction of PP1 and GSK-3 β in JBC recently.

Peter Klein: Scott, we published a paper recently in JBC on PP1 dephosphorylation of serine 9 [9].

Scott Brady: Gabrielle, there may be a variety of pathways to misregulate GSK-3. For example, cyclin dependent kinase 5 (CDK5) activity is required to keep GSK-3 off. We also have evidence that tau may play a role in this.

Gabrielle Strobel: Are any of the several CDK5, tau, or GSK mouse models suitable to addressing the question of when axonal transport begins to change?

Jorge Busciglio: Yes, Gabrielle, there are several excellent experimental paradigms to study axonal transport *in vivo*. . . optic nerve metabolic labeling, sciatic nerve ligations, etc.

Frank LaFerla: Jorge, your study on PS1 mutations altering GSK-3 β and kinesin phosphorylation states was done on embryonic neurons, correct? If so, does this not mean that disruption of axonal transport is a very early event?

Jorge Busciglio: Frank, we used embryonic neurons that differentiate in culture; it is difficult to say when the impairment in transport starts.

Changiz Geula: Scott, what is the evidence for the participation of tau?

Scott Brady: The evidence for tau is several-fold and some of it is unpublished. However, GSK-3 will bind to tau, and in some conformations, we have evidence that tau filaments can directly activate it.

Jorge Busciglio: First, let me tell you that post-mortem intervals may have confounding effects on kinase/phosphatase activities in AD brains.

Akihiko Takashima: Jorge, yes, GSK-3 β activity is impossible to determine in postmortem tissues because serine 9 is extremely labile.

Nikolaos Tezapsidis: Kinesin-driven anterograde fast and slow axonal (and to a lesser degree dendritic?) transport may be equally important as dynein-driven retrograde dendritic (and to a lesser degree axonal) transport of both cargo vesicles and multimeric proteins.

Jorge Busciglio: Yes, Nikolaos. What is the evidence for altered retrograde transport in AD?

Nikolaos Tezapsidis: Jorge, there is not much; we have started doing some studies. What I was suggesting is that we should look into it more.

Jorge Busciglio: Absolutely, Nikolaos, we will be looking at transport in KIm146V cultured neurons.

Nikolaos Tezapsidis: Jorge, perhaps we can collaborate.

Changiz Geula: Jorge and Nikolaos, disruption of retrograde transport in AD would be consistent with a host of anatomical abnormalities. Most neurotrophins get to the cell body via retrograde transport.

Jorge Busciglio: Yes, Changiz, and as you know, there are a number of motor abnormalities in AD patients.

Scott Brady: Nikolaos and Changiz, regarding retrograde, the GSK-3 effect is purely on anterograde transport, but we have just submitted a manuscript with the Sisodia laboratory that shows a decrement in the transport of Trk [tyrosine receptor kinase] receptors with FAD PS1 mutants. This will lead to reduced levels of receptors to bind neurotrophins and return them to the cell body.

Peter Klein: Jorge and Scott, have you tried any GSK-3 inhibitors (other than the CREB peptide) to test whether they affect kinesin light chain phosphorylation or fast axonal transport?

Jorge Busciglio: Peter, how does GSK-3 α affect amyloid- β protein precursor (A β PP) processing?

Peter Klein: Jorge, we are working on it. We do not know yet, but it does not appear to be through a direct effect on gamma-secretase activity.

Akihiko Takashima: Jorge, according to Peter's paper, a decrease in GSK-3 β would affect GSK-3 α activity and vice versa.

Scott Brady: Peter, we were aware of your paper, but I was referring to a pathway we have defined that involves PP1 in neurons.

Akihiko Takashima: Gabrielle, does anybody know if axonal transport is inhibited in GSK-3 β transgenic mice?

Gabrielle Strobel: Akihiko, I do not know, but I think it ought to be checked.

Diego Forero: I want to contribute a recent reference that shows alterations in axonal transport in AD patients *in vitro* [10].

Jorge Busciglio: Peter, are there GSK-3 α consensus sites in nicastrin, Pen-2, or Aph-1?

Peter Klein: Jorge, not in Pen-2 or Aph-1—not sure about nicastrin.

Changiz Geula: Jorge and Scott, in terms of the relationship between disruption of anterograde transport and A β , the disruption should cause accumulation of A β PP in the cell and thereby result in increased intracellular A β . Is this your interpretation? Do you see increased A β in your cultured cells?

Jorge Busciglio: Yes, Changiz, that is part of our hypothesis. A recent paper by Hoshi et al. [11] also shows that A β neurotoxicity increases GSK-3 activity, so it could be a positive feedback loop. PS1 mutations increase A β production, and the Met146Val is no exception.

Gunnar Gouras: Jorge, we tended to see A β accumulation in distal processes—how could this fit in?

Jorge Busciglio: Gunnar, decreased transport of mitochondria may affect processing at the tips.

Akihiko Takashima: Jorge, this was demonstrated by our group in 1993–1994.

Nikolaos Tezapsidis: Scott and Jorge, we have shown that the FAD mutations on PS1 are associated with a tighter binding of PS1 to CLIP-170, and that the two proteins interact prior to PS1's cleavage and presumably prior to its incorporation into the gamma-secretase multimeric high molecular weight complex. Whether the tighter binding of PS1/A β PP vesicles at the microtubule tip facilitates the assembly process of this complex, which generates A β , or whether it makes it harder for the cargo to move towards a degradation pathway (endosomes) remains to be determined.

Scott Brady: Nikolaos, we need to know more about the nature of the binding of GSK-3 to PS1 before we can make a connection with the CLIP-170 result.

Nikolaos Tezapsidis: Scott and Jorge, the constant motion of cargos is partially regulated by the ability of those motors to attach both to the cargo vesicles and the microtubule track. CLIP-170 is believed to serve such a regulatory role, acting as a linker of endosomes to the plus end of the microtubules.

Scott Brady: Nikolaos, the role you propose for CLIP-170 has been suggested, but not demonstrated directly. We do not see much evidence for this kind of regulation of kinesin-based motility in neurons.

Nikolaos Tezapsidis: Scott, our data are consistent with the initial proposed role for CLIP-170; however, we are the first to explore this in pathways related to AD.

Gabrielle Strobel: Nikolaos, would it be possible to do your experiments in primary neurons, in addition to neuroblastoma lines?

Nikolaos Tezapsidis: Gabrielle, that, we were planning to do next.

Peter Klein: To anyone, do the FAD mutations in PS1 affect GSK-3 binding?

Jorge Busciglio: Peter, we will be mutating the binding domains to answer that question. Takashima's results indicate that PS1 mutations indeed affect binding.

Akihiko Takashima: Peter, we did it. It seems to be the same affinity, regardless of the mutation.

Jorge Busciglio: . . . or maybe not?

Akihiko Takashima: Jorge, sorry, I just checked my PNAS paper [12]. You were right.

Akihiko Takashima: Everybody, there were questions proposed to be discussed in the chat by Jorge and Scott. Could we go through them one by one?

Diego Forero: Dr. Busciglio, what are the results with NT2 cells transfected with PS1 constructs? I ask because your recent article in *J Neurosci* [13] did not show the effects on neurite outgrowth in differentiated hNT neurons.

Gabrielle Strobel: As far as I know, GSK-3 has been a potential drug target for a long time, but the work never really took off. Is this now changing with Peter's, Akihiho's, and Jorge/Scott's work?

Peter Klein: Gabrielle, GSK-3 has been a drug target for quite a while because of tau phosphorylation as well as potential applications in diabetes and bipolar disorder. I hope attention is rekindled on targeting GSK-3, especially α , to inhibit A β PP processing.

Gabrielle Strobel: Peter, perhaps your experiments would lend themselves to the development of a suitable assay for compound screening?

Akihiko Takashima: Peter, according to your paper, inhibiting either GSK-3 will affect the other, so how can you have a GSK-3 α -specific inhibitor?

Peter Klein: Akihiko, that is an excellent point. However, we do not actually know the magnitude of GSK-3 inhibition based solely on the phosphorylation, since we do not know what fraction of total GSK-3 is phosphorylated. It might be possible to inhibit GSK-3 α without completely inhibiting GSK-3 β . Gabrielle, I agree. It would be great to identify GSK-3 α -specific inhibitors.

Gabrielle Strobel: Peter, I am asking also because Alzforum is thinking about what sorts of information resources we could compile to support academic researchers who are venturing into drug discovery, or at least target validation, themselves.

Jorge Busciglio: Peter, how specific is kenpaullone?

Nikolaos Tezapsidis: Peter, lithium can be used. . . also has been used for other brain disorders (bipolar disorder and schizoaffective disorder).

Peter Klein: Nikolaos, yes, perhaps lithium can be used, as it is for bipolar disorder. A drug with fewer side effects would be preferable in an older population, though.

Gabrielle Strobel: Peter, this has nothing to do with the fact that many AD patients have psychotic symptoms, however? Wrong?

Gunnar Gouras: Jorge and Scott, could you explore more whether A β PP/A β are intermediates or unnecessary for your PS/GSK effects?

Scott Brady: Peter, as you point out, there are other mechanisms for regulating GSK-3 activity, including various binding proteins. Much remains to be understood about the microscopic regulation of GSK-3 in different cell types.

Jorge Busciglio: Peter, again, how does kenpaullone look from a therapeutic point of view?

Peter Klein: Jorge, kenpaullone does not distinguish between GSK-3 α and GSK-3 β , and also inhibits CDKs. However, in a study from Phil Cohen's laboratory (University of Dundee, Scotland), where they examined many protein kinases, there was no overlap between lithium-sensitive kinases (which, in any event, required significantly higher concentrations of lithium) and kenpaullone-sensitive kinases.

Jorge Busciglio: Peter, are you planning lithium treatment and behavioral experiments in the mouse model?

Diego Forero: I believe there are many things to study with lithium as an inhibitor of GSK-3. There are many unresolved questions

Changiz Geula: Jorge and Scott, what is the magnitude of the presenilin effect on axonal transport? To be a therapeutic target, the effect must be shown to be substantial.

Jorge Busciglio: Changiz, the effect might be chronic and subtle, and impair neuronal homeostasis in the long term. I am not sure you need a substantial effect; that may not be compatible with survival.

Scott Brady: Changiz, as we discussed in the paper, modest changes in transport may have long-term consequences by reducing the efficiency of the transport and leading to increased vulnerability of the neuron. Transport declines with age and, therefore, the ability of the neuron to maintain itself is reduced. The PS1 effect reduces that still further. The longtime course for development of pathology suggests that relatively modest changes in transport may be highly significant.

Peter Klein: Scott, right you are. Jorge, kenpaullone is being studied as an anticancer therapy, but I do not know offhand if it has been used clinically or what its overall toxicity is.

Akihiko Takashima: Jorge, we are trying to publish a paper (with great difficulties) demonstrating that lithium can rescue the A β -induced tau pathology and memory loss in tau transgenic mice.

Peter Klein: Jorge, we are studying behavior in lithium-treated mice right now, but more from our interest in bipolar disorder.

Diego Forero: Dr. Takashima, this paper you are talking about sounds very interesting.

Akihiko Takashima: Diego, thank you. We talked about it in the last AD conference.

Gabrielle Strobel: Dr. Takashima, very interesting. Are there differences of lithium effect in tau-A β PP double transgenic versus just A β PP transgenic? This could separate effects of lithium on A β generation versus GSK3/tau.

Akihiko Takashima: Gabrielle, we are doing it. Our results so far are with A β injection.

Gabrielle Strobel: Oh, you use your tau transgenics and inject A β to make the pathology worse? That is interesting, too.

Akihiko Takashima: Gabrielle, yes, as published by Gotz in Science [14].

Nikolaos Tezapsidis: Gunnar, A β can be accumulated within neurons at the synapses either by deficient export or hyperactive import if it comes down to kinetics. Alternatively, a mere overproduction/delivery to the neuronal tips or deficient neuronal clearance. Jorge, Scott, any comments on the similarity of knockout and mutant PS1 effect versus the amyloid cascade hypothesis.

Jorge Busciglio: Nikolaos, knockout and PS1 mutants' similar effect suggest that mutations impair the ability of PS1 to keep GSK-3 β activity down (at least when kinesin light chain is the target). In that sense, amyloid production may be subjected to a different type of regulatory mechanism.

Nikolaos Tezapsidis: Jorge, this would be consistent with a loss of beneficial function for PS1. This agrees with our data derived from the neuropathological examination of AD brains that showed preservation of neurons with PS immunoreactivity.

Jorge Busciglio: Nikolaos, that is a very interesting observation.

Nikolaos Tezapsidis: Jorge, this raises another very interesting point in this post-genomic era. Proteins and their biological activities, which ultimately confer the clinical phenotype, are much more difficult to study. A higher level of creativity and ingenuity than technology can offer will be required.

Martha Stokely: Scott, perhaps you would like to comment on the possibility of multiple insults to axonal transport in addition to aging.

Jorge Busciglio: Martha, many different insults may converge in transport deficits, and aging may be one of the primary factors affecting transport by increases in oxidative stress and energy deficits.

Scott Brady: Martha, diseases of aging always involve compromises in a wide range of cellular activities. Peter, at the time we did the study, we lacked the tools to test that rigorously, but the availability of GSK-3 α -specific probes will allow us to see whether there is a difference. I suspect that both will be able to phosphorylate kinesin light chain. It is probably differential regulation and differential compartmentation that determines whether GSK-3 α or β is responsible.

Peter Klein: Scott and Jorge, have you looked at GSK-3 α phosphorylation in PS knockout or knockin settings? And do you know if GSK-3 α interacts with or phosphorylates kinesin light chain?

Diego Forero: Jorge, and what about the other binding proteins of PS1 (Notch, cadherins) and axonal transport? Have you looked for it? It would be very interesting.

Jorge Busciglio: Diego, we have not looked into that yet.

Nikolaos Tezapsidis: Jorge, and even though we should in a "funny" way consider ourselves lucky to have in our hands the FAD-linked mutations, it is still debatable whether we have a safe target.

Jorge Busciglio: Nikolaos, I agree.

Gabrielle Strobel: Many A β PP transgenics are now being made available by Jackson labs. Alzforum has links to that resource.

Jorge Busciglio: Dr. Takashima, have you worked further on the PS1-GSK-3 β binding region?

Akihiko Takashima: Jorge, unfortunately, no. We focus on tau.

Gabrielle Strobel: How could the relative roles of A β generation and transport disruption by FAD PS1 be assessed? Video microscopy of FAD transgenic mouse neurons? What other methods are suitable?

Jorge Busciglio: Yes, Gabrielle, video microscopy on living cultured neurons is what we plan to do to directly answer that question.

Diego Forero: The most specific and sensible available approach.

Gabrielle Strobel: Jorge, can this be combined with clever calcium imaging agents? I wish more people incorporated those in their AD-related microscopy.

Jorge Busciglio: Gabrielle, also classic transport studies *in vivo* will be important to determine the nature of the transport defect.

Gabrielle Strobel: Can confocal in living mice reveal such things? Or injecting labeled stuff and measuring how much got to the target in mouse models at different time points?

Diego Forero: If you consider Dai et al., 2002 [10], it will be possible to do transport studies in patients with PS1 mutations.

Jorge Busciglio: Gabrielle, calcium imaging may be important since PS1 mutations appear to deregulate calcium homeostasis. I think there may be ways we could use both techniques simultaneously.

Gabrielle Strobel: Let us address another question. Here is number 1: The amyloid cascade hypothesis still draws majority support in the field as the predominant, if incomplete, explanation for AD. Can we integrate the axonal transport and the amyloid hypotheses? Do they fit together and how?

Diego Forero: The new amyloid hypothesis (amyloid and synaptic plasticity) could be related with axonal transport very easily.

Leo Kim: Diego, could you please explain more on the topic?

Diego Forero: Hi, Leo, as shown by Kamenetz et al., 2003 [15], amyloid is a key regulator of synaptic function, and as shown by Busciglio et al. [7], changes in PS1 and axonal transport regulate the transport of A β PP.

Nikolaos Tezapsidis: Gabrielle, our paper [16] also supports that notion. We have observed a reduction in A β production and A β uptake by intercepting the interaction of PS1 with CLIP-170, a microtubule-interacting protein, implying transport.

Jorge Busciglio: Gabrielle, I think that both the amyloid hypothesis and the impaired axonal transport are perfectly compatible and may both be related. It is clear that A β will impair transport, and impaired transport may enhance A β production.

Scott Brady: Gabrielle, axonal transport helps to establish a steady state for a wide range of neuronal functions. Disruption of transport alters those homeostatic mechanisms. The result is that one can have misregulation of many processes as a downstream consequence.

Akihiko Takashima: To answer Gabrielle's question on amyloid hypothesis and axonal transport: Braak and Braak [17] investigated A β deposition and neurofibrillar changes in thousands of brains at different ages. According to the study of Braak, neurofibrillar changes in the entorhinal cortex precede A β deposition. Yet, neurofibrillar changes in the limbic and cortex regions follow A β deposition. The entorhinal stage (Braak stage I, II) marks normal aging, while the limbic (Braak stage III, IV) and the isocortex stages mark diseased states; and neurofibrillar changes in the entorhinal cortex occurs in 100 percent of the aged population. Thus, neurofibrillary tangles in the entorhinal cortex occur during brain aging, and A β accelerates NFT formation in the limbic and isocortical regions, resulting in AD. Therefore, disruption of axonal transport might occur during aging and before A β deposition because of tau dysfunction.

Changiz Geula: Dr. Takashima, would you equate disruption of axonal transport with tangle formation? Can these be independent processes?

Akihiko Takashima: Changiz, from *in vitro* and *in vivo* studies, tau accumulation in the cytoplasm disrupts axonal transport by the inhibition of association between kinesin and microtubule. Free tau is preferable to phosphorylated.

Jorge Busciglio: Dr. Takashima, do you think that tau phosphorylation precedes detachment of the microtubule?

Akihiko Takashima: Jorge, yes, I think that tau phosphorylation impairs its binding to microtubule.

Scott Brady: Gabrielle, the age of onset and slow development of pathology may mean that the two phenomena are not directly related. The experiments that

Jorge proposes will help get at that. As I noted above, there may be many things going awry because of the disruption of homeostatic mechanisms in the neuron. This is why the correlation between some pathological features and some of the clinical symptoms is poor. A more detailed analysis of transport will help with this, as Jorge says.

Diego Forero: I am in accordance with Scott, Gabrielle, as shown by Stamer et al., 2002 [18], manipulations that alter the axonal transport in a nonspecific manner have profound effects on cell function.

Gabrielle Strobel: Scott, this appears to call for quite nonspecific therapeutics aimed broadly at maintaining/restoring normal transport flows. Is anything like that conceivable today?

Scott Brady: Gabrielle, in the case of AD, the various strategies for modulating GSK-3 activity may be helpful. The key is not to eliminate activity. These kinases have too many functions for that to be a good idea. The key may be to adjust basal activity while not disrupting other regulatory mechanisms. I think this is, in principle, possible, but will require a better understanding of GSK-3 regulation and compartmentation.

Peter Klein: Scott, I agree completely with your view that much more information on subcellular distribution of GSK-3 α and β is needed.

Keith Crutcher: Folks, I have been following the discussion with some interest and confusion. I am not a transport aficionado, but I am having a hard time understanding the underlying hypothesis and how this relates to the vast majority of AD cases. Do any of the results suggest that transport defects are a cause rather than a consequence of AD pathology? Is there a testable hypothesis here?

Jorge Busciglio: Keith, synaptic dysfunction, dystrophy, and neuronal loss can all be the direct result of transport defects.

Akihiko Takashima: Jorge, yes, I agree.

Keith Crutcher: Jorge, fair enough, but why would one expect to see the regional pathology of AD from such changes?

Jorge Busciglio: Keith, many insults including A β toxicity and tau mutations may converge in transport

deficiency. Some neurons are more vulnerable than others.

Keith Crutcher: Jorge, yes, I can accept that, but I am wondering what hypothesis we can generate to pull these observations together.

Jorge Busciglio: Keith, the regional pathology may also be related with differences in A β processing in distinct brain regions.

Changiz Geula: Keith, the pathology of AD, at least tangle pathology, seems to affect primarily neurons with very long axons, which also happen to be connected with the cortex. Transport defects would be consistent with problems in long axons. However, widespread cortical connectivity appears to be another predisposing factor.

Keith Crutcher: Changiz, good point, although there are neurons with even longer projections such as Betz neurons which I do not think show major changes. . . or am I wrong on that?

Changiz Geula: Keith, you are correct. But Betz cells take a very direct route and have very little cortical connections (as least in terms of their axons). This is where widespread cortical connectivity comes in.

Scott Brady: Keith, the hypothesis is that reduction of fast anterograde transport by misregulation of GSK-3 in neurons is indeed underlying most, if not all, AD cases. There are a number of pathways that can influence these kinases. The regional specificity can arise in any one of several ways. There may be differences in the regulation of GSK-3 between neuronal populations. There may be compensatory pathways developed differentially in different neurons. Alternatively, neurons vary in their dependence on neurotrophin support and activity. Some neurons may just live closer to the edge in GSK-3 sensitive pathways.

Diego Forero: Keith and Jorge, the hippocampal cells, may have a greater dependence on axonal transport, because their greater synaptic plasticity burdens them.

Scott Brady: Keith, the diversity of the nervous system is such that for virtually any insult, neuronal populations exhibit differential vulnerability. This is seen in spinal cord injury and ischemia, as well as toxic treatments and neurological diseases like AD.

Keith Crutcher: Yes, interesting idea... suggesting that it is the total axonal arbor and/or amount of turnover?

Gabrielle Strobel: I sense a consensus here that we are not at a point where therapy development can begin in earnest, except for established targets such as presenilin and GSK-3 β . GSK-3 α seems to be a new target that has people intrigued. We have reached the end of the hour. I want to thank you all very much for coming and making this such a lively discussion.

References

- [1] G. Morfini, G. Pigino, U. Beffert, J. Busciglio and S.T. Brady, Fast axonal transport misregulation and Alzheimer's disease, *Neuromolecular Med* **2** (2002), 89–99.
- [2] Alzheimer Research Forum, Lithium Hinders A β Generation, Buffing Up GSK as Drug Target, <http://www.alzforum.org/new/detail.asp?id=817>, 22 May 2003.
- [3] G. Morfini, G. Szebenyi, R. Elluru, N. Ratner and S.T. Brady, Glycogen synthase kinase 3 phosphorylates kinesin light chains and negatively regulates kinesin-based motility, *EMBO J* **21** (2002), 281–293.
- [4] D. Cai, J.Y. Leem, J.P. Greenfield, P. Wang, B.S. Kim, R. Wang, K.O. Lopes, S.H. Kim, H. Zheng, P. Greengard, S.S. Sisodia, G. Thinakaran and H. Xu, Presenilin-1 regulates intracellular trafficking and cell surface delivery of beta-amyloid precursor protein, *J Biol Chem* **278** (2003), 3446–3454.
- [5] S. Naruse, G. Thinakaran, J.J. Luo, J.W. Kusiak, T. Tomita, T. Iwatsubo, X. Qian, D.D. Ginty, D.L. Price, D.R. Borchelt, P.C. Wong and S.S. Sisodia, Effects of PS1 deficiency on membrane protein trafficking in neurons, *Neuron* **21** (1998), 1213–1221.
- [6] Alzheimer Research Forum, Yet Another Molecular Partner for Presenilins Identified, <http://www.alzforum.org/new/detail.asp?id=334>, 25 November 2001.
- [7] G. Pigino, G. Morfini, A. Pelsman, M.P. Mattson, S.T. Brady and J. Busciglio, Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport, *J Neurosci* **23** (2003), 4499–4508.
- [8] S. Sato, Y. Tatebayashi, T. Akagi, D.H. Chui, M. Murayama, T. Miyasaka, E. Planel, K. Tanemura, X. Sun, T. Hashikawa, K. Yoshioka, K. Ishiguro and A. Takashima, Aberrant tau phosphorylation by glycogen synthase kinase-3 β and JNK3 induces oligomeric tau fibrils in COS-7 cells, *J Biol Chem* **277** (2002), 42060–42065.
- [9] F. Zhang, C.J. Phiel, L. Spece, N. Gurvich and P.S. Klein, Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium. Evidence for autoregulation of GSK-3, *J Biol Chem* **278** (2003), 33067–33077.
- [10] J. Dai, R.M. Buijs, W. Kamphorst and D.F. Swaab, Impaired axonal transport of cortical neurons in Alzheimer's disease is associated with neuropathological changes, *Brain Res* **948** (2002), 138–144.
- [11] M. Hoshi, M. Sato, S. Matsumoto, A. Noguchi, K. Yasutake, N. Yoshida and K. Sato, Spherical aggregates of beta-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3 β , *Proc Natl Acad Sci USA* **100** (2003), 6370–6375.
- [12] A. Takashima, M. Murayama, O. Murayama, T. Kohno, T. Honda, K. Yasutake, N. Nihonmatsu, M. Mercken, H. Yamaguchi, S. Sugihara and B. Wolozin, Presenilin 1 associates with glycogen synthase kinase-3 β and its substrate tau, *Proc Natl Acad Sci USA* **95** (1998), 9637–9641.
- [13] E.A. Grace and J. Busciglio, Aberrant activation of focal adhesion proteins mediates fibrillar amyloid beta-induced neuronal dystrophy, *J Neurosci* **23** (2003), 493–502.
- [14] J. Gotz, F. Chen, J. van Dorpe and R.M. Nitsch, Formation of neurofibrillary tangles in P301L tau transgenic mice induced by A β 42 fibrils, *Science* **293** (2001), 1491–1495.
- [15] F. Kamenetz, T. Tomita, H. Hsieh, G. Seabrook, D. Borchelt, T. Iwatsubo, S. Sisodia and R. Malinow, APP processing and synaptic function, *Neuron* **37** (2003), 925–937.
- [16] N. Tezapsidis, P.A. Merz, G. Merz and H. Hong, Microtubular interactions of presenilin direct kinesis of A β peptide and its precursors, *FASEB J* **17** (2003), 1322–1324.
- [17] H. Braak and E. Braak, Neuropathological staging of Alzheimer-related changes, *Acta Neuropathol* **82** (1991), 239–259.
- [18] K. Stamer, R. Vogel, E. Thies, E. Mandelkow and E.M. Mandelkow, Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress, *J Cell Biol* **156** (2002), 1051–1063.