Dear Colleagues,

This is a rather extraordinary chain of letters that illustrates rather than refutes the central premise Mr. Piller's reporting. What is clear from these missives is that the field's leaders must honestly confront the crisis of research integrity afflicting our field.

Let me address a few key issues:

To assert, as virtually every one of you has, that Sylvain Lesné's misguided article had no or minimal influence on the field is silly and Dr. Hardy's assertion that he never cited the study is flatly false. The Lesné article was one of the most cited basic science articles relating to Alzheimer's disease in the last 20 years and generated a series of high-impact articles in follow-up, many now retracted or corrected. Between 2020 and 2021 (the timeframe just prior to our initial reports), it was cited 256 times according to GoogleScholar – how many 15-year-old papers can claim that? One of the most-cited articles to reference Lesne's blockbuster in 2021 was a review article second-authored by Dr. Hardy -- **Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, Villemagne VL, Aisen P, Vendruscolo M, Iwatsubo T, Masters CL, Cho M, Lannfelt L, Cummings JL, Vergallo A. The amyloid-β pathway in Alzheimer's disease. Molecular Psychiatry 2021;26:5481-5503**. This was not the only time Hardy cited the Lesné article. A more contemporaneous assessment read as follows: "*A continued area of active research has been aimed at understanding the potential mechanism of Aβ toxicity. While original formulations of the amyloid hypothesis envisaged the deposited peptide as neurotoxic, it has become increasingly clear that smaller species assemblies have both behavioral and electrophysiological effects (Lambert et al., 1998; Walsh et al., 2002; **Lesné et al., 2006**).*" **Hardy J. A hundred years of Alzheimer's disease research. Neuron 2006;52:3-13.**

It is not clear to me why so much effort would be placed on the claim to have never cited this article, when it is so easy debunk. The fact that this line was selectively left out of an essentially carbon-copied version of this letter in *Lancet Neurology* is telling. The subsequent claim that Prof De Strooper only cited the Lesné article once, and that was to criticize it, is also untrue and remains in the *Lancet Neurology* version of Prof Hardy's letter.

Prof De Strooper has, in fact, cited that article numerous times. One example that stands out to me also appeared in *Lancet Neurology*:

*An important conclusion, therefore, is that the absolute amount of Aβ peptides generated seems to be less crucial than the particular type of Aβ peptide, at least in familial Alzheimer's disease linked to presenilin mutations. The recent insight that amyloid plaques or single Aβ peptides are not extremely toxic,[70] but that an ill-defined oligomeric state of Aβ peptides affects synapses and neurons, might provide an explanation for this apparent paradox.[71,72] (***72 is Lesné 2006***). **Bergmans B, De Strooper B. γ-secretases : from cell biology to therapeutic strategies. Lancet Neurology 2010;9:215-226.**

*"Recent studies have emphasized the toxic role of soluble lowmolecular-weight amyloid- (A) oligomers such as dimers, trimers, tetramers, nonamers, and dodecamers, which have all been individually identified as the main neurotoxic culprit (Lambert et al., 1998; **Lesne´ et al., 2006**; Shankar et al., 2008; Ono et al., 2009). …* Some of these studies have observed memory impairment following single brain infusion (**Lesné et al., 2006**; Shankar et al., 2008), but the toxicity of small

Aβ1–42 oligomers in vivo still needs to be established.*"* **Brouillette J, et al, De Strooper B, Luc Buee. Neurotoxicity and memory deficits induced by soluble low-molecular-weight amyloid β1-42 oligomers are revealed in vivo by using a novel animal model. J Neurosci 2012:32(23):7852-7861.** This latter citation stands out because of the frequent image duplications observed within its figures – perhaps Dr. De Strooper would be willing to review it: http://pubpeer.com/publications/0312E4D4088C8A0AC7D94ED4B301CD#1. I include a list of various papers authored by Drs. Hardy or De Strooper citing the Lesné article as an appendix to this letter.

It is only logical that Dr. De Strooper cites the 2006 Lesné article – it was a high-profile discovery and an important part of the rationale for targeting oligomers. In fact, far from announcing that the paper did not reproduce, at one point De Strooper claims to have "confirmed the behavioral effects of forward protofibrils generated from Aβ monomers", citing the Lesné article (Martins IC, et al, 2008). De Strooper has certainly been more circumspect about the limitations of Lesné's work than many people in the field, but it was far from discredited (as De Stroooper now claims) and formed part of the basis for pursuing oligomer targeted amyloid clearance as a therapeutic strategy. There is no reason to deny this plain fact, except to make the lapse in research integrity seem smaller than it is.

In the same vein, Drs. Grill and Rabinovici twice claim that our work identified "only" four vignettes of research integrity concerns in the field. This false claim suggests to me that the writers perhaps did not read Mr. Piller's book. Moreover, numerous writers claim that only one of the cases had anything to with the "amyloid hypothesis." Dr. Grill might be surprised to read details of a serious case at his home institution involving the development of a major amyloid-centric mouse model: https://pubpeer.com/search?q=LaFerla. In fact, our work identified dozens of examples of probable misconduct in labs scattered around the world. Some of the cases have been closer to the amyloid-hypothesis than others, but the assertion that they have no relevance to the hypothesis is untrue. The Zlokovic lab, including implicated articles, extensively addressed β-amyloid clearance mechanisms, Eliezer Masliah published volumes of material around the role of β-amyloid and plaques in neurodegeneration. Frédéric Checler and Peter St George Hyslop worked on presenilin and gamma-secretase function and APP processing. Even Cassava Sciences' platform, which was clearly outside of the mainstream, argued that their drug interrupted β-amyloid signaling. Importantly, not a single one of the numerous of cases we describe are isolated episodes, nor are they merely about "loading controls". Our bar for inclusion was set very very high; each case involves numerous manuscripts with very serious problems, most of them forming a sustained pattern over a decade or more and squandering enormous resources. In the span of a few years we have seen multiple clinical trials tainted by research integrity violations, several biotech companies suffer massive losses over research integrity violations, the president of a leading university step down over mishandling of the research integrity violations of others, the dismissal of the director of the division of neuroscience at the NIA, a top-ten lab hobbled, directors of major research centers step down, and editors at several journals become embroiled in controversy. This is not business as normal. We can debate to what degree this has altered the overall state of the field, but diminishing the significance of this behavior is exactly the opposite of what our field needs.

The group of assembled writers would seem to prefer that I and others stop speaking about research integrity – describing our work as less gentile than the old approach of simply ignoring these types

issues, or in Dr. Hardy's parlance equating it to a "public murder." Some writers dismissively treat our work as merely the byproduct of advancing technology which makes spotting errors and manipulation in certain types of data easier. While these tools have their place, the Lesné and Cassava Sciences cases were conducted entirely without these tools and manual forensics is a major part of all of these investigations. What has changed is not so much the technology, but that a small group of people have decided we care enough to speak publicly about this issue and put the needs of patients first. Direct and indirect threats to the careers of those who speak up, often from previously respected colleagues, tens of thousands of dollars in legal expenses and the deafening silence of prominent voices speak to the urgent need for transformational leadership in our discipline.

Dr. De Strooper's "unequivocal condemnation of fraud" is tempered by later complaining that "by bringing this matter into the public domain, the journalist has created an inherently unequal situation, where information that should have been handled with due process is now subject to selective public scrutiny." While the assertion of various writers that most Alzheimer's scientists are above reproach is certainly true and every accused party does indeed deserve due process, the tolerance of the field's leaders and key institutions of misconduct perpetuates its influence. I can attest that the current concept of "due process" in scientific spheres can at times look a lot like a coverup – in my view, the public scrutiny is warranted and necessary.

Matthew Schrag MD, PhD

Quotes from key citations:

Hardy J. A hundred years of Alzheimer's disease research. Neuron 2006;52:3-13.

"A continued area of active research has been aimed at understanding the potential mechanism of Aβ toxicity. While original formulations of the amyloid hypothesis envisaged the deposited peptide as neurotoxic, it has become increasingly clear that smaller species assemblies have both behavioral and electrophysiological effects (Lambert et al., 1998; Walsh et al., 2002; **Lesné et al., 2006**)."

Hardy J, Orr H. The genetics of neurodegenerative diseases. J Neurochemistry 2006;96:1690-1699.

"The nature of the toxic species of Aβ is also not entirely clear, but an evolving view has been that it is some form of Aβ oligomer: this evolving consensus has centred around a species called A4 in the original APP cloning paper, or more recently Alzheimer's Disease Diffusible Ligands, Aβ oligomers or Aβ* (Klein et al. 2004). Strictly, these species have not been shown to be toxic, but rather have been shown to be potent synaptic depressants (**Lesné et al. 2006**). Much of the effort for developing mechanistic therapeutics has been aimed at APP processing: by inhibiting either BACE or γ-secretase either directly or indirectly (Hardy and Selkoe 2002; Golde 2005). The greatest publicity has been given to trials of immunization against Aβ. This immunization

clearly and surprisingly results in the clearance of Aβ from the brains of mice and humans, and has behavioral benefits in the mice, but the human trial resulted in serious and occasionally fatal meningoencephalitis in a proportion of Alzheimer cases without clear clinical benefit (Schenk et al. 2004). Whether this was an adverse effect of the particular protocol used or was centrally related to the immunological approach to Aβ clearance is currently unclear. In the Alzheimer field there is the general sense that mechanistic approaches to therapy should emerge soon, but there is also a growing impatience amongst those who believe that Aβ-centred therapy should have worked by now if indeed it was a valid approach to the disease (Lee et al. 2005)."

Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH, Villemagne VL, Aisen P, Vendruscolo M, Iwatsubo T, Masters CL, Cho M, Lannfelt L, Cummings JL, Vergallo A. The amyloid-β pathway in Alzheimer's disease. Molecular Psychiatry 2021;26:5481-5503.

Soluble Aβ oligomers derived from human brains have molecular weight distributions corresponding to a mixture of dimers to dodecamers [168 -- ***Ashe 2013 review*, 169 – *Lesné 2013 Brain***]. Intracellular and secreted soluble dimeric and trimeric Aβ oligomers were observed in human-derived neurons, as well as APP transgenic mouse models [156, 170, 171]. Mass spectrometry studies have shown that brain-derived bioactive 7 kDa Aβ species are composed of a heterogeneous mixture of covalently cross-linked dimers of different Aβ fragments, which might represent the smallest synaptotoxic species [172, 173].

AND

Soluble protofibrils of various sizes have been identified in human brains and in brains from APP transgenic mice [191–193, ***192 is Lesné 2006***].

Benilova I, Karran E, De Strooper B. The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. Nature Neuroscience 2012;15(3):349-357.

"Indeed, apart from the Aβ dimers and trimers34, larger Aβ oligomers and structures such as the amylospheroids (ASPD), which are spherical Aβ assemblies of 10–15 nm36,37, have been isolated from Alzheimer's-affected brain tissue and suggested by investigators to be the elusive toxic Aβ oligomeric species postulated above. Experiments using brain tissue from transgenic mice have yielded very controversial results. In the J20 mouse model of Alzheimer's disease, which expresses human APP containing two familial mutations, no clear correlation between memory deficits and the presence of dimers, trimers or other Aβ species could be established38. The authors concluded: "These data demonstrate the presence of multiple assembly forms of Aβ throughout the life of J20 mice and highlight the difficulty in attributing synaptotoxicity to a single Aβ species". In Tg2576 transgenic mice that overexpress Swedish-mutated human APP, a 56-kDa species called Aβ*56 was correlated with cognitive deficits39 **– Lesné 2006**. Purified Aβ*56 caused memory deficits when injected in brains of young rats. An important concern with Aβ*56 is the presence of 0.1% SDS in the initial extraction buffer39 because, as discussed, SDS might artificially promote the aggregation of Aβ24 and could also be a matter of concern when assessing toxicity in cellular or in vivo assays. Overall, the experiments present a very mixed picture: they generally support the existence of a mixture of water-soluble Aβ species that exert synaptotoxicity, but it remains to be clarified whether this toxicity can be specifically associated with dimers, trimers or other assemblies, and whether these assemblies can mediate neurotoxicity in Alzheimer's disease. Probably the most crucial question is the nature and biological relevance of the SDS resistance of the identified dimers, trimers and larger oligomers. If it is a property induced by the presence of SDS, then it is unlikely to be meaningful with respect to the putative pathophysiological role of toxic oligomers.

If it can be demonstrated, however, that additional covalent modification is responsible for the conversion of Aβ monomers to these in vivo dimers, and if this could be unequivocally correlated with toxicity, then their relevance would be indisputable."

Karran E, Merchen M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nature Reviews Drug Discovery. 2011;10:698-712

A modification of the amyloid cascade hypothesis is supported by increasing evidence from the literature that smaller, soluble oligomeric species of amyloid-β mediate either neuronal death or affect synaptic neurotransmission**113–115. (114 is Lesné 2006)** This is an attractive concept, as it neatly resolves the issues mentioned earlier regarding the lack of correlation among deposited plaques, tau pathology and neuronal loss. Thus, oligomeric or dimeric amyloid-β might diffuse through the brain parenchyma and mediate neuronal stress or perhaps cause direct toxicity. However, this remains a highly complex area of science and there are several issues that remain to be clarified.

De Strooper B. Proteases and proteolysis in Alzheimer's disease: a multifactorial view on the disease process. Physiol Rev 2010:90:455-494.

Over the last decade, the concept of "Aβ-derived diffusible ligands" (ADDL) (164) or "soluble toxic oligomers" (164, 173 - **Lesné**, 329) has advanced. Several different oligomeric assemblies of the Aβ peptide have been described, generated in vitro (45), or isolated from transfected CHO cells as stable dimers, trimers, and multimers (234) or from transgenic mouse brains as a 56-kDa oligomer (173 - **Lesné**). Various oligomeric species have also been isolated from the brains of AD patients; the smallest toxic isolate was reported to be comprised of a dimeric structure (275). It is quite likely that different oligomeric species are in dynamic equilibrium with each other, single peptides, and inert fibrils (250), a balance which may be influenced by the presence of lipids (195). Currently, no consensus exists with regard to which toxic Aβ assembly is most relevant in vivo, and it is not unlikely that various conformations of toxic aggregates exist next to each other.

Kuperstein I, et al, De Strooper B. Neurotoxicity of Alzheimer's disease Aβ peptides is induced by small changes in the Aβ42 to Aβ40 ratio. The EMBO Journal 2010:29:3408-3420.

A series of intermediate soluble aggregates of Aβ peptides, such as 'Aβ-derived diffusible ligands' (ADDLs) (Lambert et al, 1998) or 'natural toxic oligomers' (Walsh et al, 2002), have been identified. The mechanism of their neurotoxic activity remains not only subject of intense investigation, but also the precise conformation(s) of the toxic species remains uncertain (Kayed et al, 2003; Hepler et al, 2006). Dimers were proposed to potently disrupt synaptic plasticity (Klyubin et al, 2008; Shankar et al, 2008), an Aβ species of 56 kDa has been found neurotoxic in Tg2576 mice (**Lesné et al, 2006**), lipid-induced oligomers from mature fibrils (Martins et al, 2008), ADDLs (Lambert et al, 1998; Gong et al, 2003; Lacor et al, 2007) and annular assemblies (Lashuel et al, 2002) were shown to exert neurotoxic effects, affect synapse function and even memory formation in mice.

De Strooper B. Loss-of-function presenilin mutations in Alzheimer's disease: talking points on the role of presenilin mutations in Alzheimer disease. EMBO Reports 2007;8(2):141-146.

Since the original amyloid-cascade hypothesis for AD was put forward (Hardy & Higgins, 1992), many modifications and refinements have been proposed to incorporate new observations and to resolve apparent conflicts. For example, no absolute relationship exists between amyloid load in the brain and the clinical manifestation of AD symptoms in humans (Price & Morris, 1999) or mice (Games et al, 1995). This has led to

the concept of Aβ-derived diffusible ligands (Lambert et al, 1998) or ‘soluble toxic oligomers’ (Glabe, 2006; Lambert et al, 1998; Walsh et al, 2002). These Aβ oligomers are intermediary forms between free soluble Aβs and insoluble amyloid fibres, and seem to be toxic both in vitro and in vivo. Although the molecular nature of these oligomers remains elusive, they have been isolated from transfected Chinese hamster ovary cells (Walsh et al, 2002) and as a 56-kDa oligomer from transgenic mouse brains (**Lesné et al, 2006**). The extent to which PSEN1 mutations generate mixtures of Aβs that are more prone to form toxic oligomers remains to be investigated; however, this concept could explain cases of AD in which smaller amounts of Aβ are generated.

Martins IC, et al, De Strooper B (co-last author/corresponding), Schymkowitz J, Rousseau F. Lipids revert inert Aβ amyloid fibrils to neurotoxic protofibrils that affect learning in mice. The EMBO Journal 2008:27:224-233.

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by neurofibrillary tangles and amyloid plaques consisting of aggregated Aβ-peptide (Hardy, 2002). Fifteen years ago, the ‘amyloid hypothesis’ for AD has been proposed (Selkoe, 1991; Hardy and Higgins, 1992), but the discrepancies between amyloid plaque load in the brain and cognitive impairment in the patient (Price and Morris, 1999) or mice (Games et al, 1995) have caused a lot of controversy in the field (Terry, 2001). This has led to the concept of ‘protofibrils’ (Harper et al, 1997; Walsh et al, 1997, 1999; Hartley et al, 1999), ‘annular assemblies’ (Lashuel et al, 2002; Bitan et al, 2003); ‘Aβ-derived diffusible ligands’ (Lambert et al, 1998) or ‘soluble toxic oligomers’ (Podlisny et al, 1995, 1998; McLean et al, 1999; Walsh et al, 2002a; Glabe and Kayed, 2006). These species are intermediary forms between free soluble Aβ-peptides and insoluble amyloid fibers and are toxic in vitro and in vivo, whereas mature Aβ-amyloid fibers are largely inert (Aksenov et al, 1996). The molecular nature of these smaller assemblies of Aβ remains rather elusive (Hepler et al, 2006), as different sources, isolation procedures and biophysical techniques lead to different conclusions. A number of species have been observed: dimeric and trimeric Aβ-oligomers (Podlisny et al, 1995, 1998; McLean et al, 1999; Walsh et al, 2002a), 56* kDa oligomer assemblies from transgenic mouse brains (**Lesné et al, 2006**) or larger structures that consist of 50 and more Aβ peptides (Lambert et al, 1998; Walsh et al, 1999; Nilsberth et al, 2001; Chong et al, 2003; Barghorn et al, 2005; Wogulis et al, 2005; Hepler et al, 2006; Haass and Selkoe, 2007).

……..

Our results confirm the behavioral effects of forward protofibrils generated from Aβ monomers (Hartley et al, 1999; Walsh et al, 2002a; Kamenetz et al, 2003; Cleary et al, 2005; Klyubin et al, 2005; **Lesné et al, 2006**; Townsend et al, 2006) and show that lipid-induced protofibrils generated from mature Aβ fibrils have very similar pathophysiological effects.

Brouillette J, et al, De Strooper B, Luc Buee. Neurotoxicity and memory deficits induced by soluble low-molecular-weight amyloid β1-42 oligomers are revealed in vivo by using a novel animal model. J Neurosci 2012:32(23):7852-7861.

Recent studies have emphasized the toxic role of soluble low-molecular-weight amyloid-β (Aβ) oligomers such as dimers, trimers, tetramers, nonamers, and dodecamers, which have all been individually identified as the main neurotoxic culprit (Lambert et al., 1998; **Lesne´ et al., 2006**; Shankar et al., 2008; Ono et al., 2009). Given the rapid oligomerization of Aβ, some authors have suggested that toxicity could be due to A species ranging from 10 to 100 kDa present in the AD brain at the same time, rather than to just one particular type of oligomer (McLean et al., 1999; Hepler et al., 2006; Martins et al., 2008). Thus far, the neurotoxic effect of low-molecular-weight Aβ oligomers was tested exclusively on primary neuronal cultures or organotypic brain slices since no appropriate animal model is currently available to determine the neurodegenerative effect of Aβ

species in vivo (Lambert et al., 1998; **Lesné et al., 2006**; Hung et al., 2008; Shankar et al., 2008). Some of these studies have observed memory impairment following single brain infusion (**Lesné et al., 2006**; Shankar et al., 2008), but the toxicity of small Aβ1–42 oligomers in vivo still needs to be established.

Bergmans B, De Strooper B. γ-secretases : from cell biology to therapeutic strategies. Lancet Neurology 2010;9:215-226

An important conclusion, therefore, is that the absolute amount of Aβ peptides generated seems to be less crucial than the particular type of Aβ peptide, at least in familial Alzheimer's disease linked to presenilin mutations. The recent insight that amyloid plaques or single Aβ peptides are not extremely toxic,[70] but that an ill-defined oligomeric state of Aβ peptides affects synapses and neurons, might provide an explanation for this apparent paradox.[71,72] **(72 is Lesné 2006)**.