Author response

Response to reviewer 1

[…] it is clear they believe pharmacological manipulation of wnt signaling is a viable approach. So could they maybe include a section summarizing the studies of consequences of chemical or genetic manipulation of wnt signaling in different neural cell types or experimental animals, and could they discuss which are the promising candidates (if any). Some of this information can be gleaned from other sections but putting it into a concise section would be interesting.

We have added a table (Table 1) that summarises the various chemical/genetic manipulations of Wnt signalling that have been reported in different neural cell types or experimental animals (page 17). With an outlook on potential future therapeutic avenues in mind, we restricted Table 1 to those approaches where the goal was to increase or restore Wnt signalling in order to achieve an improved therapeutic or favourable outcome with respect to ameliorating AD or neurological pathology.

We further discuss the potential for future therapeutic development in the modified Conclusion section (Section 5 (lines 993-1071).

Minor point. In the studies being reviewed, could they make it clear which cell types are the focus of the study. This is only clear in a few examples.

We have updated the manuscript and provided the information requested by reviewer 1. We have tried to indicate specific cell types (where known) throughout the text. We have added a final section that specifically discusses opportunities and challenges for chemical or genetic manipulation of Wnt signalling that could have potential therapeutic utility in the future.

Response to reviewer 2

1. The use of the word "deregulated" in the article title is not the proper term; "dysregulation" is. Deregulated means "unregulated" which is not the case of the Wnt pathway in Alzheimer's disease. In fact, canonical Wnt signaling in AD is suppressed which is quite the opposite of "deregulation." The correct term to use is dysregulated, which means "dysfunctional or aberrant regulation" and more clearly describes what is happening to this signaling pathway in AD.

We have replaced the term "deregulated" with "dysregulated" throughout the manuscript.

2. The first paragraph should grab the reader's attention about the significance of the paper, and why this review (among all the other literature reviews on this topic) is significant and adds new

information/interpretation to the current state of the research in the field. The writing in this paragraph sounds "choppy" and fails to grab attention. In line 25, replace the term "carers" with "caregivers", as the former is not as common of a term as the latter. Thus, I would recommend rewording that entire sentence to read ... "profoundly impacts caregivers, families, and society as a whole" ...to better represent AD's significance. Also, the last sentence (lines 30-32) seems to have some syntax errors and should be rewritten to flow more smoothly.

We have replaced the term "carers" for "caregivers" and have reworded the designated section of the sentence to read "profoundly impacts caregivers, families, and society as a whole", as recommended (lines 31-32). We further modified the last sentence of this paragraph (originally lines 30-32) for easier readability. We have also rewritten the abstract to hopefully grab the reader's attention, and more clearly spell out what the review will cover and what is aims to achieve.

3. There are numerous published manuscripts with elegant visual depictions of Wnt signaling cascades and it might be best to replace Figure 1 with a reference for one of these previous works. Figure 1, as presented, is cumbersome with too many detailed labels using small font and doesn't clarify the pathway any better than the written description. The goal of a figure is to summarize and enhance what is written in the text—this figure does not accomplish this. Given that so many other manuscripts do provide graphic representations of normal Wnt signaling pathways, the authors would be better off creating a figure that highlights the specific elements of Wnt pathways that are shown to be altered in AD, as this would support the review's purpose and be informative to the reader. Therefore, the recommendation is to either remove this figure or replace it with something of this nature.

Figure 1 has been significantly simplified in accordance with the recommendations made by Reviewer 1 (page 3). What remains is a schematic of the Wnt pathways that appear to be most relevant in AD: canonical Wnt signalling and Wnt/PCP signalling. We removed the Wnt/Ca2+ pathway from the original figure, although a written description was retained. We further removed several of the modulators of Wnt signalling (WIF1, Cerberus, Notum, Dkk1, WISE/SOST, IGFBO-4, RNF43/ZNRF3, R-Spo, LGR4/5, and Norrin) in order to make Figure 1 less convoluted, even though written descriptions were retained. We hope the amended figure now meets with the reviewer's approval.

4. The authors spend several paragraphs describing the detailed role of Wnt signaling role in the developing brain, but this doesn't seem to provide any useful information for interpreting any of the later discussion about Wnt defects in AD. This creates a problem because all of this superfluous detail distracts from the purpose of the paper which is to link Wnt deficits with AD. While Wnt patterning is involved in hippocampal development and synaptogenesis, these facts can just be stated succinctly because the authors do not provide any details of how these organization processes are pathologically changed in AD (because they aren't necessarily). Therefore, I recommend reducing the section on Wnt signaling in developing brain into perhaps a few summarized sentences acknowledging Wnt's role in neural development. As for the sections on Wnt's role in the mature and aging brain, this information seems like it would be best re-organized and presented in context with how Wnt signaling differs or is altered in comparison to the AD brain. Are the age-related decreases in canonical Wnt signaling qualitatively different than what is seen in AD, or just quantitatively different (i.e. an exacerbating of the normal aging process). The distinction between changes associated with normal, healthy aging and the pathological disease state of AD should be emphasized.

We have followed the reviewer's advice and have very significantly shortened Section 3.1 (page 4) on Wnt signalling in brain development. We did think it to be important to retain some key elements within this section. This especially applies to lines 285-292, which describe the role of Wnt signalling with respect to synaptogenesis. Synapses are an important point of focus in AD pathology and the link to reduced Wnt signalling has been well established. We wanted to emphasise the key principle that the balance between canonical and non-canonical Wnt/PCP signalling determines whether (pre-)synaptic assembly is increased or decreased. This principle is recapitulated in AD where evidence suggests that a pathological shift from canonical to Wnt/PCP signalling contributes to synapse destabilisation and loss (see lines 768-787 in Section 4.3).

Regarding Wnt signalling in the ageing versus the AD brain, as per the reviewer's recommendation to consider this aspect we have now added a new section (Section 4.6, lines 951-995). We highlight that commonalities between these two states exist, one of them being the upregulation of *Dkk1* in aged mice and in AD mouse models. However, while *DKK1* was also found to be upregulated in human AD patients, no data is presently available for humans undergoing normal ageing. Besides just *DKK1*, there is a profound scarcity of published literature on Wnt signalling changes in general during normal ageing, especially in humans, and a gap in our understanding of the molecular mechanisms that underlie changes in the Wnt system. Hence a detailed analysis regarding putative quantitative or qualitative differences in Wnt signalling between normal ageing and AD was not possible at the time of writing. Nonetheless, we hope that we sufficiently addressed the reviewer's question by highlighting what is known, the gaps in our knowledge, and opportunities to address them.

5. The statement written in lines 310-311 about "boosting" Wnt signaling should be rewritten because it comes across as oversimplified: does this mean "restoring" deficient signaling or "enhancing" basal signaling? The act of enhancing Wnt signaling is no small endeavor given that most therapeutics are designed to suppress it (i.e. anti-cancer drugs), and the statement oversimplifies the complexity of determining which of the many mechanisms of the Wnt cascade are most strategic to manipulate in terms of enhancing/restoring Wnt signaling.

We agree with the reviewer and have replaced "boosting" with "restoring (line 536). Thereafter, we also added sentences explaining that any potential approaches that target Wnt signalling need to be carefully considered given the multiple targetable Wnt pathway components and given the potential side-effects such an approach might bear, one of them being tumour formation (lines 535-543). Additionally, we have re-written Section 5 to consider the opportunities and challenges of modulating Wnt signalling in a therapeutic context.

6. While Figure 2 has the title "Deregulated wnt signaling in AD," there is no description of which specific Wnt signaling elements are associated with each of these pathological problems of AD and therefore, the figure is uninformative, and should be revised accordingly. Also change the term "deregulated" to "dysregulated" in any revisions of the figure.

We have amended Figure 2 (page 9) to now include broad graphical descriptions on the nature of known or potential Wnt signalling changes (the latter were indicated with "?") using arrows to indicate whether increased or decreased Wnt signalling is evident or might be expected.

Additionally, the term "deregulated" has been changed to "dysregulated".

7. Its unclear what message the authors are trying to convey with what is written in lines 460-464 about frontotemporal dementia. This distinction between FTD and AD here doesn't seem relevant to the introduction of the topic of tauopathy. Also, the statement made in line 465 is outdated and not supported by current research in the field, or even by the more recent papers cited by the authors. The overall consensus of research in the field does not consider tau pathology to be necessary for amyloid-beta pathogenesis in either human clinical cases or rodent models. In fact, there is more evidence that amyloid beta facilitates the development of tauopathy, although this topic itself is contentious and amyloid-independent mechanisms for tauopathy pathogenesis have also been identified. Therefore, the authors should reword this statement/section to reflect the fact that there are important mechanistic linkages between amyloid beta and tau but the precise nature of the relationship is complex.

We recognise the useful advice by the reviewer and have modified this paragraph accordingly. FTD was briefly mentioned in this paragraph because we had just described in the preceding Section 4.1 how mutations in *APP*, the proteolytic products of which are major components of amyloid plaques, can cause FAD. In the light of this, we thought it worth mentioning that mutations in *TAU*, the major component in NFTs, do not cause FAD although one might expect that to be the case, but do lead to another type of dementia (FTD) (line 677-681).

Although mutations in *TAU* and *APP* can lead to different types of dementia, there still exist mechanistic links between them that are yet to be fully described when just focusing on AD. We fully agree with the reviewer in that point and we have modified this paragraph to reflect this (lines 681-685).

8. In lines 497-498, the authors state that amyloid beta formation precedes synaptic dysfunction, cognitive deficits and tauopathy, and this is also a contentious and outdated finding (the Sperling report is from 2011). Some of the earliest pathological indicators in human tissue are actually in fact tauopathy, again, underscoring the independent pathways by which each of these pathologies arise, and some of the most recent clinical findings show that plasma or CSF levels of tau180 are the earliest biomarkers (Rodriguez + 2020: PMID: 32720099). There is nothing wrong with linking amyloid aggregation to synaptic dysfunction, but one must be careful in describing the nuances, direction, and timing of the mechanistic association between these events as it is not well-established in the field.

We have amended this section of the manuscript, as per the reviewer's recommendations (lines 724-735) and focused the paragraph on the relationship between synapses and the emergence of Aβ.

9. There are numerous reviews on Wnt signaling deficits in AD, and the authors should strive towards the goal of distinguishing this review as unique and useful to someone who investigates this

topic (such as myself). As such, the discussion/conclusions section of a review should be a call for action based on the data that has been reviewed and what is clearly still needed to move this field forward. This section seems to fall short of this. The authors mention concepts such as "early detection" of Wnt deficts in AD but fail to expound upon why/how this can be accomplished and what work still needs to be done. While other sections of this manuscript provide too many details, this section does not provide enough detail to drive home the importance of what this review of information on Wnt signaling in AD tells us about the state of the field.

Based on the comments provided by the reviewer we have significantly modified the discussion/conclusion (Section 5, lines 696-766) and now entitle it "Looking forward: does Wnt signalling offer opportunities for new therapies for AD?". In this section we consider opportunities and challenges for therapeutic modification of the Wnt signaling system in AD, with some specific examples. We also highlight some key gaps in our knowledge that need to be filled to enable such therapeutic approaches. We believe that this re-focussed section aligns with the reviewer's suggestion, and importantly will be of interest to readers of the review.