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External examiner report on the PhD thesis submitted by Andrea Špolcová titled:
'Impact of different types of antidiabetic interventions on the development of neurodegenerative changes in brains of diabetic mice and rats.'

By Prof. Christian Hölscher, PhD

The thesis submitted by Andrea Špolcová describes the investigation of the effects of novel prolactin-releasing peptide analogues in animal models of diabetes and in a transgenic mouse that expresses a human mutated tau gene.

The student has produced a lot of data of good quality. The write-up of the work is also at a high linguistic standard. Three papers have been published that are based on the work of the student, documenting the quality of the work. Generally speaking, I think that the work is of the standard of a doctorate degree.

I have several questions concerning the concepts and interpretation of the data presented in the thesis.

The introduction is extremely short. The main point of an introduction is to prepare the reader for the specific experiments that had been carried out in the PhD project. The scientific background is explained, and the previous work that has been published in the field is summarised and put into context.
The introduction that has been submitted gives a good overview of Alzheimer's disease and the two common biomarkers, the plaques and tangles. Type II diabetes and obesity, which really is the main focus on the thesis, is not mentioned other than as a risk factor for AD. The majority of the work is carried out in animal models of diabetes and obesity, and the standard biomarkers of diabetes are investigated (see title, and the Aims of the thesis on page 36). The drugs tested are primarily directed against diabetes and obesity. An overview of what diabetes is, and what the drugs tested actually do in the periphery and in the hypothalamus to reduce appetite would be suitable.
Next, a brief overview of animal models of diabetes and Alzheimer's is presented. The animal models that actually have been used in the PhD project should be described in much more detail. The Thy-tau22 model has only been described in a short paragraph. What genetic background does this mouse model have? We are not told this important piece of information anywhere. Where does the tau mutation come from, what is

frontotemporal dementia with Parkinsonism? What symptoms does this induce? Afterwards, this model is described as a model for Alzheimer's disease. However, there is no tau mutation associated with Alzheimer's disease. What previous work has been done with this mouse model? What phenotype does it have? What is the literature on this? We do not know if it is impaired in motor activity, which would affect the results of the study.
Furthermore, we are given no information what receptor these PrRP peptides activate, and what the cellular second messenger signalling cascades are. As the studies investigate levels of kinases that are involved in second messenger signalling cascades, this is really of central importance as will become clear later on. Some information on how these peptides affect appetite would also be helpful.
There are other points to be raised.

The Materials and Method chapter is detailed. There is only one memory task, a Y maze task. There is no rationale given why this task was chosen, nor why the author assumes that this is a spatial task. No motor activity task has been done to check that motor activity is normal in the tau tg mice (there is a big difference in body weight between tg and wt animals!). No anxiety test has been conducted to test for differences. Anxiety can influence memory tasks, and would bias the choice of arms. Why wasn't the number of arm entries measured?
Why were there no other memory tasks, or at least different versions of the Y-maze task (eg. spontaneous alternate arm visits)?

The different results chapters are well written, but there are some questions of presentation. The statistical results are not given in detail. Only p-values are shown, no F values or n numbers. Two-way ANOVAs have been used, but the individual results have not been mentioned at all (there are two results for each Two-way ANOVA). In some graphs, there are no statistical results mentioned at all (eg. fig. 14B, p48).
Fig 27, page 65: A student t-test is used for analysing 9 different groups. This is a classic error. A one-way ANOVA has to be used for more than two groups.
Fig 28, page 66: Why is there such a big difference in body weight between tg and wt mice? It is not caused by a difference in food intake. Does this not indicate major physiological differences between the groups, which can explain the behavioural differences?
P65: only % of time spent in arms is shown. Do the groups differ in movement and in arm entry numbers?
There are additional questions on details.

The discussion is very short and consists mainly of repeating the results. There are a lot of open questions. It is very superficial and makes statements that suggest that the student has no real understanding of the subject matter. For example:
P73: GSK-3ß is described as 'the main kinase implicated in the insulin signaling cascade', yet insulin receptor activation *reduces* GSK-3ß activity. On page 22, there is a brief sketch of the insulin cascade, which shows a number of other kinases. Also, the western blot analysis on page 50 lists a series of kinases that are linked to insulin signaling. Perhaps the student does not really understand what kind of cell signaling cascade the insulin receptor actually activates?
We are told several times that the mechanism of neuroprotection by these peptides is unknown. In fact, there is a lot known about them. Numerous studies have analysed the effect on cell metabolism and glucose utilisation, gene expression and insulin re-sensitisation, reduction of oxidative stress, improvement of mitochondrial activity,

blocking of apoptosis, and a lot more. It appears that the student has little knowledge if these key studies. This impression is supported by the statement on page 79:
"....there is the possibility that palmitoylated peptides are anorexigenic, and regarding our study with MSG mice, it seems that both peptides [PrRp and liraglutide] have a similar mechanism of action."
Attaching palmitate to a peptide does not make it anorexigenic, and the fact that the student speculates that PrRp and liraglutide have a similar mechanism of action shows that she does not know what these drugs actually do. We have very detailed knowledge about what kind of receptor is involved in the actions of both peptides, and what cellular signaling cascades are activated by them, and what the downstream effects are. I am surprised that this student spent more than 3 years working with a peptide without asking herself what exactly the cellular mode of action of the peptides in her study is.

I will ask these and other questions in the oral examination.

Yours,

Prof. Christian Hölscher, PhD