Uncovering CNS assess of lipidated exendin-4 analogues by quantitative whole-brain 3D light sheet microscopy

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Background & Aim

Peptide-based drug development for CNS disorders is challenged by poor blood-brain barrier (BBB) penetrability of peptides. While acylation protractions (lipidation) have been successfully applied to increase circulating half-life of therapeutic peptides, little is known about the CNS accessibility of lipidated peptide drugs. Light-sheet fluorescence microscopy (LSFM) has emerged as a powerful method to visualize whole-brain 3D distribution of fluorescently labelled therapeutic peptides at single-cell resolution.

Methods

Functional assay
High throughput time-resolved fluorescence cAMP kit was used to measure intracellular cyclic adenosine monophosphate (cAMP) upon incubation of hGLP1-1R CHO-K1 cells with the IR800-labelled peptides.

In vivo experiments
Mice received an intravenous dose of IR800-labelled Ex4, Ex4_C16MA or Ex4_C18DA. One group was dosed with Ex9-39_C16MA, serving as negative control for GLP-1R mediated agonist internalization. For 3D biodistribution study, mice were terminated 120 min post-dosing, while for the functional assays, blood glucose and plasma insulin levels at 15 min and 120 min post-dosing.

3D imaging and image analysis
Brains were cleared using the iDISCO+ protocol and imaged using light sheet microscopy (LSFM). Region delineation of the whole-brain samples were obtained by atlas segmentation. Group averages and standard deviations were calculated for each voxel, resulting in p-value calculations compared to the vehicle group.

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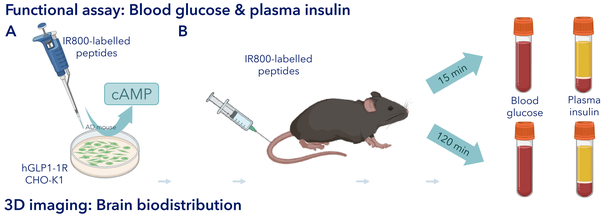
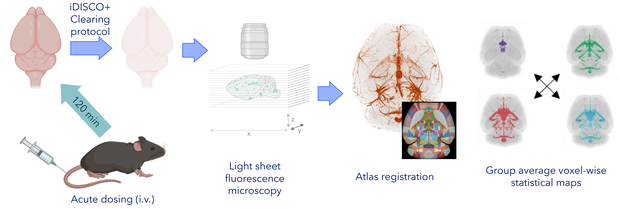
1 Methods

Functional assay: Blood glucose & plasma insulin



3D imaging: Brain biodistribution



Group	Number of animals	Treatment	Administration route	Dosing concentration
Ex4^{800}	5	Ex4^{800}	IV	100 nmol/kg
Ex4_C16MA^{800}	5	Ex4_C16MA^{800}	IV	100 nmol/kg
Ex4_C18DA^{800}	5	Ex4_C18DA^{800}	IV	100 nmol/kg
Ex9-39_C16MA^{800}	5	Ex9-39_C16MA^{800}	IV	100 nmol/kg

2 Generation of lipidated fluorescent Ex4 peptides

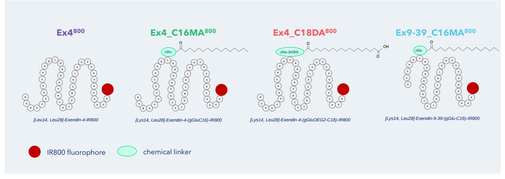


Figure 1. Generation of fluorescently labelled Exendin 4 peptides. Illustration of the peptides used in the study. The lipidations are similar to the ones used in Liraglutide (C16MA) and Semaglutide (C18DA).

3 In vivo efficacy of Ex4 peptides on BG and plasma insulin

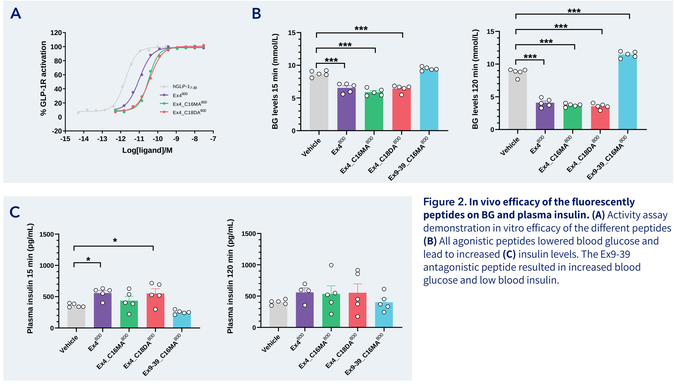


Figure 2. In vivo efficacy of the fluorescently peptides on BG and plasma insulin. (A) Activity assay demonstration in vitro efficacy of the different peptides **(B)** All agonistic peptides lowered blood glucose and lead to increased **(C)** insulin levels. The Ex9-39 antagonistic peptide resulted in increased blood glucose and low blood insulin.

4 In vivo distribution of Ex4 peptides in the CNS

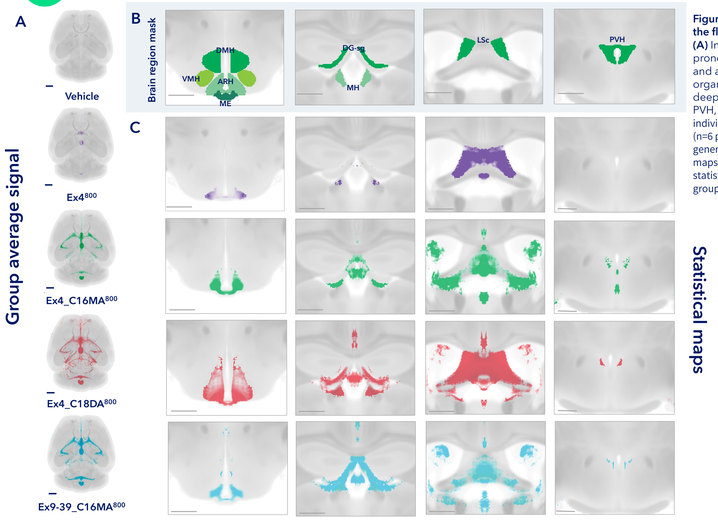


Figure 3. In vivo distribution of the fluorescent peptides in CNS. (A) In the CNS there was pronounced signal intensity in and around the circumventricular organs, choroid plexus and in deeper brain regions such as the PVH, VMH and DMH. **(C)** Using the individual maps from each mouse (n=6 per group) it is possible to generate voxel based statistical maps. The maps show voxels the are statistical different from the vehicle group (p<0.05).

5 Voxel wise statistical maps of Ex4 distribution

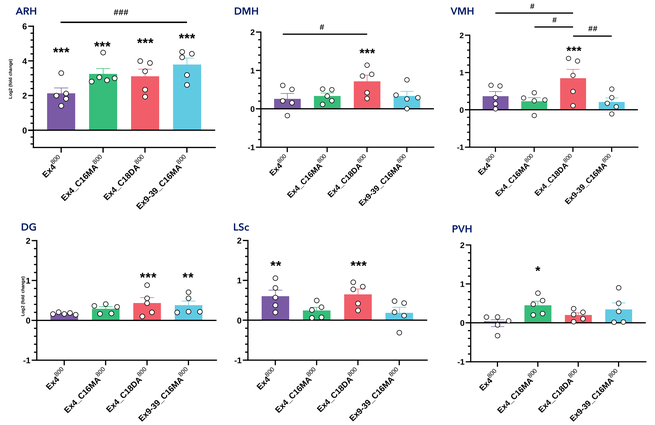


Figure 5. Accumulated signal intensities in selected brain regions. In most of the brain regions the C18DA lipidation lead to increased signal intensity. However, in the PVH the C16MA displayed a stronger signal than the C18DA.

Conclusion

- We have developed a high-throughput 3D imaging deep pipeline for brain-wide analysis of drug distribution
- Using voxel based statistical maps we were able to detect differences in brain distribution of similar peptides
- Our pipeline can be used to study the distribution of fluorescently labelled peptides, antibodies and ASO's

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