Characterization of the glucagon-like peptide-1 receptor in male mouse brain using a novel antibody and in situ hybridization

Glucagon-like peptide-1 (GLP-1) is a physiological regulator of appetite and long-acting GLP-1 receptor agonists (GLP-1RA) lower food intake and bodyweight in both human and animal studies. The effects are mediated through brain GLP-1Rs, and several brain nuclei expressing the GLP-1R may be involved. To date, mapping the complete location of GLP-1R protein in the brain has been challenged by lack of good antibodies and the discrepancy between mRNA and protein especially relevant in neuronal axonal processes. Here, we present a novel and specific monoclonal GLP-1R antibody for immunohistochemistry with murine tissue and show detailed distribution of GLP-1R protein expression as well as mapping of GLP-1R mRNA by non-radioactive in situ hybridization. Semi-automated image analysis was performed to map the GLP-1R distribution to atlas plates from the Allen Institute of Brain Science (AIBS). The GLP-1R was abundantly expressed in numerous regions including the septal nucleus, the hypothalamus and the brain stem. GLP-1R protein expression was also observed on neuronal projections in brain regions devoid of any mRNA which has not been observed in earlier reports. Taken together, these findings provide new knowledge on GLP-1R expression in neuronal cell bodies and neuronal projections.

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Authors: Jensen, C. B. (Intern), Pyke, C. (Ekstern), Rasch, M. G. (Ekstern), Dahl, A. B. (Intern), Knudsen, L. B. (Ekstern), Secher, A. (Ekstern)
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Quantitative evaluation of peptide analogue distribution in mouse tissue using 3D computer modelling

The use of automated image analysis of microscopy images is increasing to enable high throughput approaches and unbiased analysis of the increasingly large data sets produced. This thesis investigates the use of automated image analysis to quantify peptide analogue distribution in mouse brain tissue. The main group of peptides included in this work was glucagon-like peptide 1 receptors agonists (GLP-1RA) used for treatment in diabetes and obesity. Two main image modalities have been applied for image acquisition; Light Sheet Fluorescence Microscopy (LSFM), and slide scanner images of 2D histology sections. The work demonstrates the use of automated image analysis based on image registration to quantify LSFM data of the peptide brain distribution following peripheral administration. The methodology was expanded during the PhD work to also include study of receptor mapping and brain activation. The automated analysis was enabled by integration with a digital multimodality brain atlas from the Allen Institute of Brain Science (AIBS). The work showed that GLP-1RAs accessed multiple brain regions mainly in the hypothalamus and hindbrain and led to increased brain activation in regions related to decreased food intake. The developed integrated brain atlas provides a novel analysis approach for LSFM data to aid researchers understand the complex brain biology related to development of pharmaceuticals with brain mode of action.

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Active Appearance Segmentation for Intensity Inhomogeneity in Light Sheet Fluorescence Microscopy

Active Appearance Models (AAM) are used for annotating or segmenting shapes in biomedical images. Performance relies heavily on the image data used to train the AAM. In this paper we improve the generalization properties of the model by making it robust to slowly varying spatial intensity inhomogeneities which are often seen in Light Sheet Fluorescence Microscopy (LSFM) images. This robustness is achieved by modelling the appearance of an image as a regularized Normalized Gradient Field (rNGF). We perform two experiments to challenge the model. First it is tested using a repeated leave-one-out approach on images with minimal imperfections where the left out images are corrupted by a simulated bias field and segmented using the AAM. Secondly we test the model on LSFM images with common acquisition problems. In both experiments the proposed approach outperforms the often used AAM implementation based on Sum of Squared Differences.

Quantification of Brain Access of Exendin-4 in the C57BL Mouse Model by SPIM Fluorescence Imaging and the Allen Mouse Brain Reference Model

With the recent advance in 3D microscopy such as Single Plane Illumination Microscopy (SPIM) it is possible to obtain high resolution image volumes of the entire mouse brain. These data can be used to study the access of several peptides such as the glucagon-like peptide-1 (GLP-1) analogue Exendin-4, into the brain with the aim of developing medication for obesity. To investigate mode of action of the medication it is important to identify the specific anatomical brain nuclei that are targeted by the compound. Such segmentations can be obtained using an annotated digital brain atlas. We construct a SPIM brain atlas based on the Allen mouse brain 3D reference model and use it to analyze the access of peripherally injected Exendin-4 into the brain compared to a negative control group. The constructed atlas consists of an average SPIM volume obtained from eight C57BL mouse brains using group-wise registration. A cross-modality registration is performed between the constructed average volume and the Allen mouse brain reference model to allow propagation of annotations to the SPIM average brain. Finally, manual corrections of the annotations are performed and validated by visual inspection. The study shows that Exendin-4 have access to brain regions such as the arcuate hypothalamic nucleus and the nucleus of the solitary tract, which are areas involved in regulating food intake.
Quantitative evaluation of peptide analogue distribution in mouse tissue using 3D computer modelling

Technical University of Denmark
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Number of participants: 6
Phd Student:
Jensen, Casper Bo (Intern)
Supervisor:
Conradsen, Knut (Intern)
Main Supervisor:
Dahl, Anders Bjorholm (Intern)
Examiner:
Dyrby, Tim Bjørn (Intern)
Kirik, Deniz (Ekstern)
Nielsen, Mads (Ekstern)

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