Decoding Neurobiological and Genetic Mechanisms of Obesity Through Big Data and Cross-Species Studies of Brain Structure and Function

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Background: Brain structure and function are coupled to eating behaviour and obesity. This coupling involves bi-directional relationships that encompasses the brain's control of appetite and the feedback loop of physiological processes triggered by feeding behaviours on the brain(*1*, *2*). When these are out of balance, it can lead to obesity and set off a cascade of inflammatory and other processes that affect the brain's structure and function.

The impact of obesity on the brain and conversely the brain's contribution to obesity have been studied in separate silos. One silo consists extensive research on obesity and metabolic disorders in humans, relying on clinical outcomes, cognition, and brain MRI(*3*). In the other silo there are detailed mechanistic experiments in rodents, with invasive cellular and molecular outcomes investigated(*4*). These latter studies give us access to mechanism (*5*), but the disparity in outcome variables between human and model animal work, coupled with the simplifications inherent in animal studies, makes reconciling the human and animal model silos difficult.

Our proposal is to bridge the gap between the silos by leveraging MRI studies in both rodents and humans. By having a readout of the brain that is directly comparable between mice and humans, we can quantitatively assess which aspects of obesity in humans are captured by rodent models. Where the translatability between human and rodent MRI is high, the cellular and molecular information that can only be captured in the mouse can be applied to the human more reliably.

Overall aim of the project: To understand the molecular processes by which obesity affects the brain. To this end this fellowship project will rely extensively on existing human and a mix of existing and newly acquired mouse data.

Aim 1 (human data): On the human side, reanalyses of the UK Biobank (UKB) and the ABCD (Adolescent Brain Cognitive Development) study will provide well powered data on how obesity impacts brain structure and function. We will emphasize regression clustering in testing associations between BMI, blood glucose levels, etc., and brain imaging measures. Crucially, this does not assume that a single brain-obesity pattern exists but rather allows for several such relations. We will follow the regression clustering algorithm developed in our recent study of social behaviour(*6*), wherein a Gaussian mixture model is used to cluster participants using a given brain measure and a given obesity related variable. Next, linear regression analysis will be used to test the brain-obesity associations within each cluster. Each time a significant regression coefficient is found, the respective entry in a feature-participant matrix will be incremented, and hierarchical clustering applied, with bagging and permutations used for stability and validity.

Having established regression clusters forming distinct brain-obesity association we will test what separates the individuals in each clusters. We will perform both univariate and multivariate analyses of cluster differences in demographic factors (age, sex, income, etc.) and, critically, execute a genome wide association study to identify variants associated with each cluster. Enrichment analyses will then be carried out to understand the molecular pathways that contribute to different brain-obesity associations.

Deliverables: Aim 1 will deliver clusters of participants with distinct brain-obesity associations and genetic variants and pathways underlying each cluster.

Aim 2 (mouse data). On the mouse side, we will acquire a new dataset where wild-type mice will be fed varying diets and imaged with high-field MRI to assess concomitant changes in brain structure and function. Brain areas showing significant changes will then be further assayed with transcriptomics. We have also collected three datasets to date that will supplement the newly acquired data. In one of the studies adult mice were fed either normal or high fat diets, and followed from 2 to 5 months of age with serial anatomical MRI(7). At the 5 month timepoint single nucleus RNA-seq and Serial Two Photon Tomography for microglia were carried out. The other two studies, one published(*7*) and the other in preparation (see figure 1), focused on the effects of maternal obesity on offspring brain and behaviour. Diets varying in calories and fat content were given to the mothers, and the offspring then followed from the early post-natal period into adulthood. We will then map between the human regression clusters and the four mouse MRI datasets using a common space approach(9) relying on common patterns of brain connectivity(10) and/or the expression of homologous genes(*11*) across the speciesWhere a human regression cluster matches experimental mouse data we will then, based on cell and molecular data from the mouse experiment, infer biological pathways likely to underly the human results. These will be taken to aim 3.

Deliverables: Putative biological pathways/genes via mouse to human alignment.

Aim 3 (i*n vitro* modelling): Here we will perform invitro modelling of cellular phenotypic changes to identify causal genes. After computational identification of a panel of potentially causal genes involved in brain function and diet, we will attempt to identify directionality of causation *in vitro*. To do so, genes will be clustered based on cell-type specific expression profiles, and neuronal targets will be brought forward for functional testing iPSC-derived POMC neurons. We will perform CRISPR-Cas9 driven gene editing in the neuronal cells for the target genes, and run a battery of phenotypic readouts on the derived KO lines comparing low glucose, to an *in vitro* nutritional overload model (4.5mM glucose, 200uM palmitate, compared to 1mM glucose). This will include morphology assessment using the ML and microscopy teams at NNRCO, combined with cytokine production – linking inflammatory readouts with cellular morphological changes. Hits which show clear functional changes will be subjected to bulk RNA-sequencing to identify the transcriptomic consequences of the gene deletion, and taking the first step towards a functional genomics approach to understanding how microglial activity during obesity drives neuronal changes.

Deliverables: The data produced here (functional readouts, brightfield imaging, cytokine section, and RNA-seq) will be integrated to provide a core picture of how specific genes linked to changes in brain function in obesity affect neuronal phenotype.

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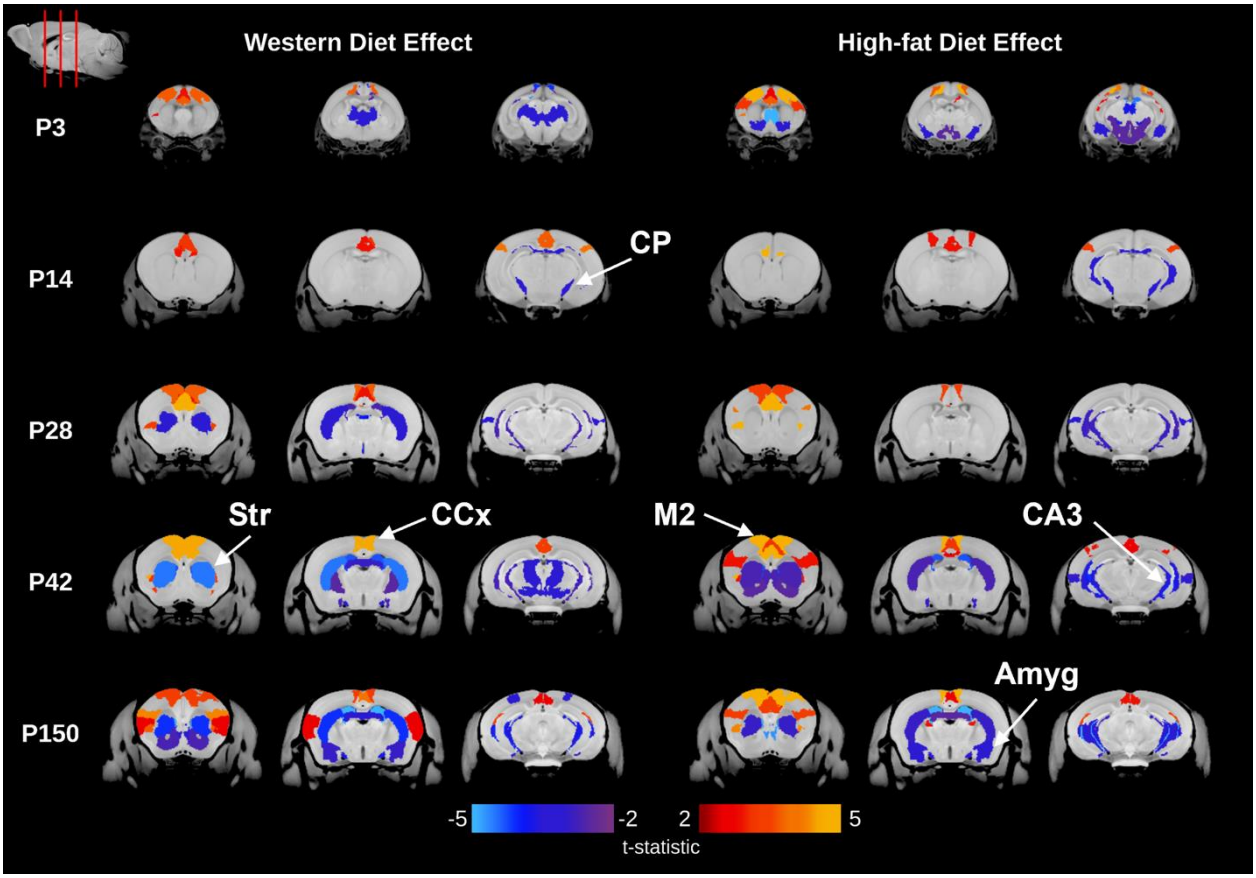

Figure 1: Gestational and early-life consumption of Western Diet or High Fat Diet was found to affect the relative volume of several brain structures (21 and 20 structures at P42, respectively) from neonatal period to adulthood. Each column follows a coronal cross-section of the developing brain through the experimental timepoints down the rows. Warm colours indicate structures relatively larger in experimental diet groups and cool colours indicate structures relatively smaller in experimental diet groups. Statistics were thresholded to 10% FDR. T-values greater than 5 were clamped by the colour bar for display purposes. The slice indicator on the top left corner shows the location of the coronal cross-section slices. CP: cerebral peduncle. Str: striatum. CCx: cingulate cortex. M2: secondary motor cortex. CA3: hippocampal CA3 region. Amyg: amygdala.

Supervisor's recent relevant publications:

Prof Jason Lerch (h-index: 104, Citations: 45,290; *Source: Google Scholar accessed on January 25, 2025*):

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2. Patel, Y., Woo, A., Shi, S., Ayoub, R., Shin, J., Botta, A., Ketela, T., Sung, H. K., **Lerch, J.**, Nieman, B., Paus, T., & Pausova, Z. (2024). Obesity and the cerebral cortex: Underlying neurobiology in mice and humans. *Brain, behavior, and immunity*, *119*, 637–647. https://doi.org/10.1016/j.bbi.2024.04.033
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Prof Rogier Mars (h-index: 66, Citations: 18,720; *Source: Google Scholar accessed on January 25, 2025*):

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3. **Mars, R. B.**, Jbabdi, S., & Rushworth, M. F. S. (2021). A Common Space Approach to Comparative Neuroscience. Annual review of neuroscience, 44, 69–86. https://doi.org/10.1146/annurev-neuro-100220-025942
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Dr Kamila Szulc-Lerch (h-index: 23, Citations: 2,065; *Source: Google Scholar accessed on January 25, 2025*):

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