Project title: Somatosensory networks control glucose homeostasis

Background. According to the canonical view, glucose homeostasis is achieved via crosstalk between peripheral signals (e.g., nutrients, hormones), in the bloodstream, and **a central regulatory system,** in the brain. The brain integrates these signals and modulates pancreatic activity via the autonomic nervous system (ANS) (Fig.1). Disruption of this control system can lead to diabetes, a major health issue[1]. The mechanisms underlying glucose homeostasis regulation and dysregulation are not fully understood. While it is acknowledged that the brain is an important regulator of blood glycemia, a crucial role for the somatosensory nervous system (SNS) in the control of homeostasis is now emerging. Somatosensory neurons, whose cell bodies are in dorsal root ganglia (DRG), innervate the pancreas[2]. While most DRG neurons form ascending circuits with the brain (i.e. spinal neurons, Fig. 1, in pink), a subset of somatosensory neurons forms visceral reflex arc networks[3,4], similar to the well-known somatic reflex arcs, with pre- and post- ganglionic autonomic motor neurons located in the spinal cord and sympathetic ganglia (i.e. Arc neurons, Fig.1 in orange). The identity and function of the neurons forming these networks are not well understood. **We hypothesize** that spinal and Arc networks innervating the pancreas are molecularly and functionally independent, crucial components of a **peripheral regulatory unit** controlling glucose homeostasis (Fig. 1), and that the disruption of these networks contributes to disease onset and progression. In support of the notion that these networks participate in glucose homeostasis is the fact that a subset of DRG neurons expresses the insulin receptor, *InsR*[5], suggesting that somatosensory networks can sense changes in the pancreas microenvironment. We believe that the characterization of these circuits, which might work in parallel or synergy with the central regulator systems[6], will radically transform our understanding of the neuron-organ interactions controlling glucose homeostasis and help to identify new peripheral targets to develop therapeutic strategies against diabetes and co-morbid conditions, such as obesity.

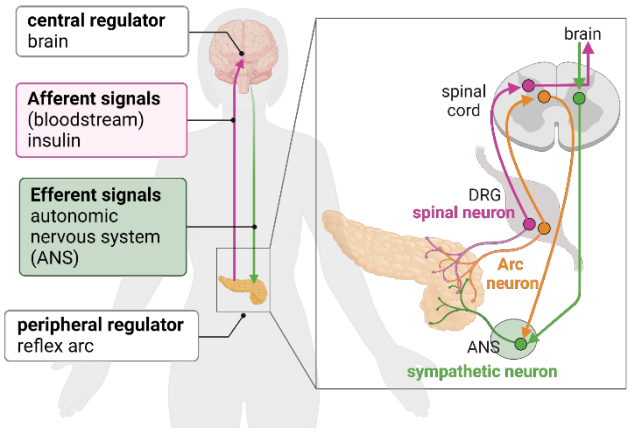

Figure 1. Proposed organization of spinal (pink) and Arc somatosensory networks (orange) innervating the pancreas and regulating glucose homeostasis. DRG: dorsal root ganglion; ANS: autonomic nervous system. Created with Biorender.com

Objectives:

1. to molecularly identify the pancreatic spinal and Arc somatosensory networks
2. to interrogate their role in the regulation and dysregulation of glucose homeostasis

Methodology:

1. Viral tracing of the pancreatic spinal and Arc networks – *in situ* hybridization or immunohistochemistry for sensory molecular markers.
2. Viral vector-based ablation of somatosensory pancreatic networks – interrogation of changes in glucose homeostasis, morphology, and gene expression of pancreatic tissue.

Work plan (including a description of the work the postdoc fellow will perform)

Objective 1. The postdoc will use a dual viral tracing approach to identify spinal and Arc neurons and unveil their molecular profile. Spinal and Arc neurons projecting to the pancreas can be traced, in the same animal, with a dual labeling approach described previously[3]. The pseudorabies virus (PRV) and

the Herpes simplex virus type H129 (H129-HSV1) are polysynaptic viral tracers, spreading across synapses. Once injected into the pancreas, the H129-HSV1 virus, conjugated to the red fluorescent protein RFP, will propagate anterogradely and label afferent DRG neurons (Fig.1, in pink). The PRV virus, instead, will propagate retrogradely and express the green fluorescent protein GFP in motor neurons in the pre- and post-synaptic sympathetic ganglia, and the spinal RFP-traced DRG neurons forming, with these neurons, the visceral arcs (i.e., Arc neurons, Fig. 1, orange neurons). With this approach, the postdoc will be able to visualize RFP^+ spinal neurons and RFP^+GFP^+ Arc neurons. To define the molecular identity of these two cell types, the postdoc will carry out fluorescent in situ hybridization (FISH) or immunohistochemistry for sensory markers that we and others identified by single-cell sequencing, including the receptor for insulin, *InsR*[7,8]. Because of their distinct anatomical organization, we expect that spinal and Arc neurons will express a distinct array of molecular markers. However, we anticipate that the insulin receptor will be expressed in both spinal and visceral Arc neurons.

Objective 2. The postdoc will selectively ablate pancreas-innervating somatosensory neurons, and assess changes in glucose homeostasis, morphology, and gene expression of the pancreas. To do this, transgenic mice, expressing a conditional form of *Cre* recombinase under the control of markers expressed in spinal and Arc neurons or both (e.g., $InsR^{ERT2}$ mice), will be injected into the DRGs with AAVs containing ablating (i.e., the *Cre*-dependent *AAV-DIO-Casp3-GFP* vector) or control substrates (i.e., the *Cre*-dependent *AAV-DIO-GFP* vector). Upon administration, tamoxifen will enable *Cre*-mediated expression of *Casp3* or *GFP* selectively in pancreas-projecting DRG neurons, therefore ablating or leaving unperturbed somatosensory pancreatic networks. The postdoc will use lean, **normoglycemic transgenic mice** (fed chow) to investigate whether ablation of somatosensory neurons leads to diabetes onset, and **obese, diabetic mice** (fed a high-fat diet for 12-14 weeks) as a model to test the impact of somatosensory network disruption on mice with an ongoing disease phenotype. Following ablation, the postdoc will assess changes in glucose homeostasis, behavior, and metabolism by performing glucose and insulin tolerance tests (GTT, ITT) and measuring the levels of insulin, triglycerides, free fatty acids, and high- and low-density cholesterol (HDL and LDL) in the plasma of control and experimental mice. Further, the postdoc will surgically implant telemetry devices and test mice in metabolic cages, to evaluate changes in feeding behavior, thermoregulation, and respiratory exchange rate (RER). Lastly, the postdoc will analyze changes in the morphology (Hematoxylin and Eosin) and gene expression (RNAseq) of pancreatic tissue, dissected from *GFP* and *Casp3* mice. We anticipate that the ablation of spinal and arc networks will differentially alter tissue integrity, transcriptional profiles, and glucose homeostasis in normoglycemic mice and exacerbate the diabetic phenotype in diabetic mice. These data would establish somatosensory neurons as a novel site for anti-diabetic therapy.

Relevance of project for diabetes. This project introduces a new conceptual model for the control of glucose homeostasis by somatosensory spinal and Arc networks. The results of this proposal have the potential to radically transform our understanding of glucose control in health and disease, as well as our approach to treating diabetes, by identifying novel cellular targets for therapy within the peripheral networks innervating the pancreas.

References

1. Alonge, K.M., et al., Diabetologia 64, 5-14 (2021).
2. Hampton, R.F., Jimenez-Gonzalez, M. & Stanley, S.A. Unravelling innervation of pancreatic islets. Diabetologia 65, 1069-1084 (2022).
3. Ryu, V. & Bartness, T.J. Am J Physiol Regul Integr Comp 306, R886-900 (2014).
4. Love, J.A., Yi, E. & Smith, T.G. Auton Neurosci 133, 19-34 (2007).
5. Lazar, B.A., et al., Pancreas 47, 110-115 (2018).
6. Furlan, A., et al. Nature Neuroscience (2022).
7. Furlan, A., et al. Nat Neurosci 19, 1331-1340 (2016).
8. Usoskin, D., et al. Nat Neurosci 18, 145-153 (2015).

Contact details

Name and title: Alessandro Furlan, Assistant Professor
Affiliation: The Department of Neuroscience, Karolinska Institutet, Solnavägen 9 | 171 65 Solna
Mail: Alessandro.furlan@ki.se; Phone: +46 (0)737426843
Website: https://ki.se/en/neuro/furlan-lab-neurobiology-of-obesity
Twitter: https://twitter.com/A__Furlan