3D mapping and quantification of semaglutide-induced neuronal activation: a study of mRNA and protein signatures in whole mouse brains using light sheet fluorescence microscopy

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Background & Aim

With small probes and an adaptable design, whole-brain spatial RNA imaging allows for visualization and analysis of gene expression, even for gene products that are difficult to stain using traditional antibody methods. Here, we present a streamlined whole-brain mRNA imaging pipeline that integrates *in situ* hybridization (ISH) technology and iDISCO tissue clearing. It holds promise for drug target discovery and action mechanism elucidation across various research areas. In the current study, we applied this technique to assess the CNS stimulatory effects of the GLP1R agonist semaglutide, providing an overview of c-Fos expression at both mRNA and protein levels.

Methods

Triplex ISH: whole-brain ISH labelled adult mouse brain for tyrosine hydroxylase (*Th*), dopamine transporter (*Dat*), and norepinephrine transporter (*Net*). Co-visualization of the mRNA targets was achieved through light sheet fluorescent microscopy (LSFM). Semaglutide study: chow-fed male C57BL/6J mice were administered an acute subcutaneous (s.c.) dose of semaglutide (0.04 mg/kg) or vehicle. Brains were collected 120 min post dosing and processed for whole-brain c-Fos mRNA or protein detection, respectively using whole-brain ISH or iDISCO (n=6 per group). Upon clearing, brains were scanned using LSFM and c-Fos cells count was analysed in ≥1,100 brain regions.

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1 Whole-brain triplex *in situ* hybridization protocol: revealing dopaminergic and noradrenergic cell populations

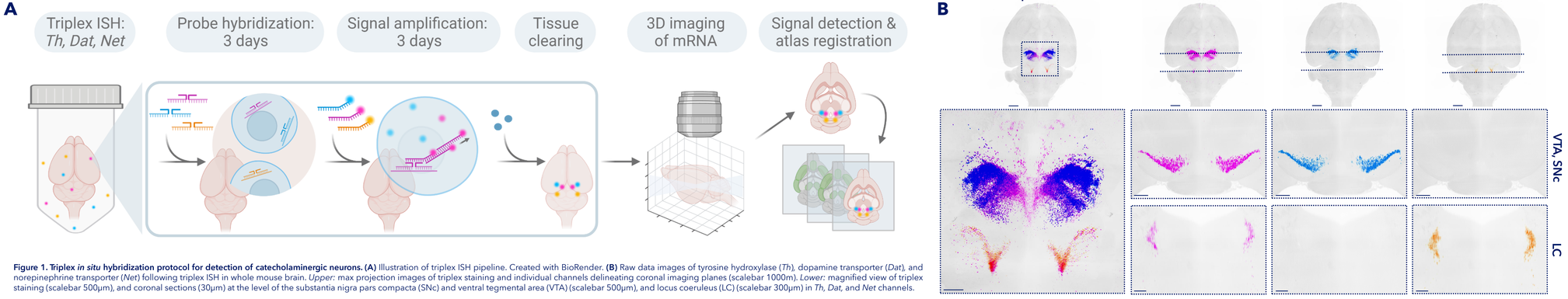

Figure 1. Triplex *in situ* hybridization protocol for detection of catecholaminergic neurons. (A) Illustration of triplex ISH pipeline. Created with BioRender. **(B)** Raw data images of tyrosine hydroxylase (*Th*), dopamine transporter (*Dat*), and norepinephrine transporter (*Net*) following triplex ISH in whole mouse brain. *Upper:* max projection images of triplex staining and individual channels delineating coronal imaging planes (scalebar 1000µm). *Lower:* magnified view of triplex staining (scalebar 500µm), and coronal sections (30µm) at the level of the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) (scalebar 500µm), and locus coeruleus (LC) (scalebar 300µm) in *Th, Dat,* and *Net* channels.

2 Paired mRNA and protein imaging of c-Fos 120 min post dosing with semaglutide

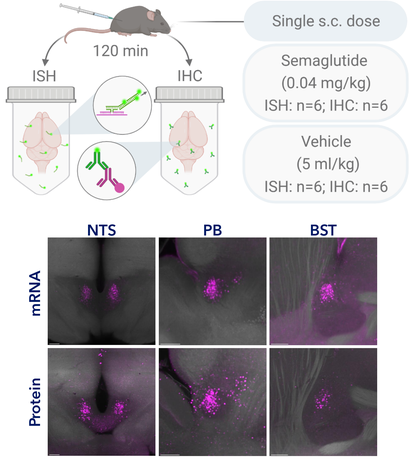

Figure 2. c-Fos expression at mRNA and protein level. *Upper:* overview of staining strategy and groups. Created with BioRender. *Lower:* raw data horizontal images (100µm) of c-Fos expression post Semaglutide dosing at mRNA (upper) and protein (lower) level in the nucleus of the solitary tract (NTS), parabrachial nucleus (PB), and the bed nuclei of the stria terminalis (BST). c-Fos signal is shown in magenta, and autofluorescence is grey. Scalebar 200µm.

3 Top 10 significant regions regulated by semaglutide relative to vehicle

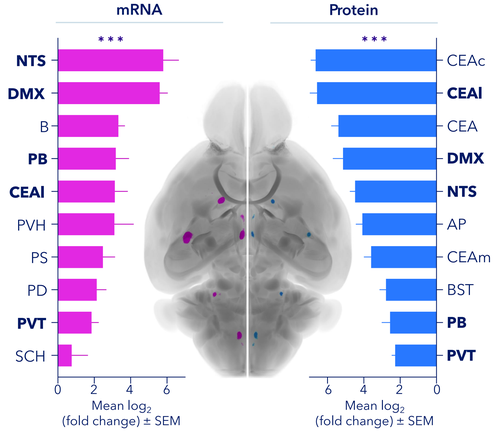

Figure 3. c-Fos activity in response to Semaglutide treatment. Heatmap representing vehicle-subtracted average c-Fos activity upon Semaglutide administration at mRNA (left) and protein (right) level. Based on region-level statistical analysis, bar charts demonstrate the top significant regions, regulated by Semaglutide relative to vehicle, ranked by fold-change. Bold regions: present in both mRNA and protein groups. ***: P<0.001.

4 Convergence of c-Fos mRNA and protein expression in key appetite-regulating regions following semaglutide administration

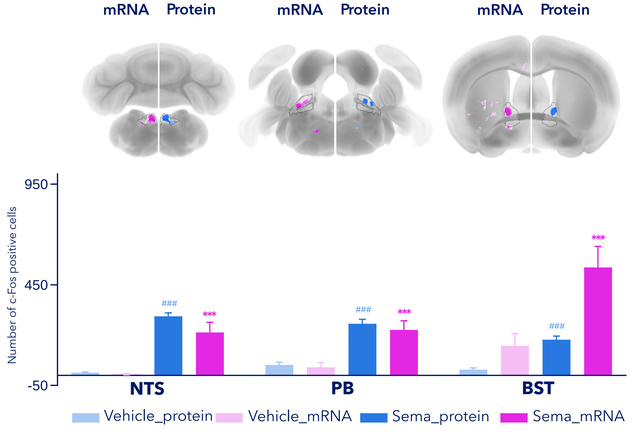

Figure 4. Appetite-related c-Fos expression hotspots. *Upper:* based on voxel-level statistics, p-value coronal section of semaglutide-induced c-Fos mRNA (left) and c-FOS protein (right) is shown at the level of the NTS, PB, and BST respectively (regions delineated in black). *Lower:* corresponding barplots showing the counts of c-Fos positive cells in the respective brain regions, for the semaglutide and vehicle-dosed mice (values expressed as mean of n = 6 + SEM; ***: p < 0.001, ###: p < 0.001; Dunnett's test negative binomial generalised linear model).

Conclusion

- Whole-brain ISH is a powerful method allowing co-visualization of multiple gene products in the intact brain
- Semaglutide induces c-Fos expression in appetite-regulating brain regions, with consistency between the staining patterns generated by protein- and RNA-targeting probes
- The incorporation of both c-Fos mRNA and protein imaging allows for better interpretation of the spatial dynamics of neuronal activation

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