# Whole-brain activation signatures of weight-lowering drugs

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## Background & Aim

The development of effective anti-obesity therapeutics relies heavily on the ability to target CNS signaling mechanisms critically involved in the homeostatic control of body weight. To get insight into neurocircuits recruited by anti-obesity drug treatment, the present study aimed to determine whole-brain activation signatures of six different weight-lowering drug classes with documented efficacy in humans.

## Methods

Chow-fed C57BL/6J mice (n=8 per group) received a single dose of the following weight lowering compounds:

+ Lorcaserin, 5-HT2C receptor agonist (7 mg/kg, i.p.)
+ Rimonabant, cannabinoid CB1 receptor antagonist (10 mg/kg, i.p.),
+ Bromocriptine, D2 receptor agonist (10 mg/kg, i.p.),
+ Sibutramine, dual noradrenaline-serotonin reuptake inhibitor (10 mg/kg, p.o.),
+ Semaglutide, GLP- receptor agonist (0.04 mg/kg, s.c.) or
+ Setmelanotide, melanocortin-4 receptor (MC4R) agonist (4 mg/kg, s.c.).

Brains were sampled two hours post-dosing, immunolabelled using c-Fos expression as a proxy for neuronal activation. Cleared whole-brains were imaged using a high-throughput light sheet fluorescence microscopy-deep learning pipeline enabling fully automated 3D mapping and quantitation of brain activation patterns at single-cell resolution.

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## 1 Study outline

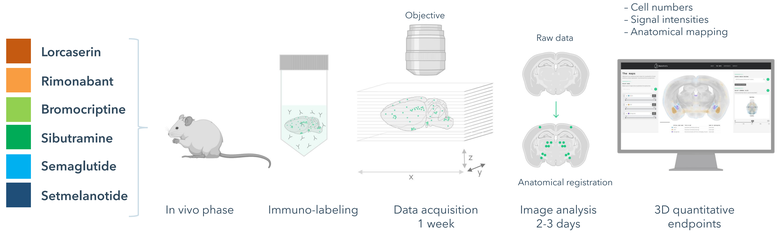



**Figure 1. Study design and groups.** Mice (n=8) were dosed with lorcasarin (7mg/kg; ip), rimonabant (10mg/kg; ip), bromocriptine (10mg/kg; ip), sibutramine (10mg/kg; po), semaglutide (0.04mg/kg; sc), setmelanotide (4mg/kg; sc) and compared to respective ip, sc, or po dosed vehicle controls. Brains removed were sampled sampled two hours post-dosing, immunolabelled using c-Fos expression as a proxy for neuronal stimulation. Brains were scanned on a Lavision Ultramicroscope II and mapped into a common reference atlas. The total number of c-Fos positive cells was quantified and the individual brains from each group were aligned into an average brain providing a heatmap of overall c-Fos activity.

## 2 3D mapping and quantification of whole-brain c-Fos responses

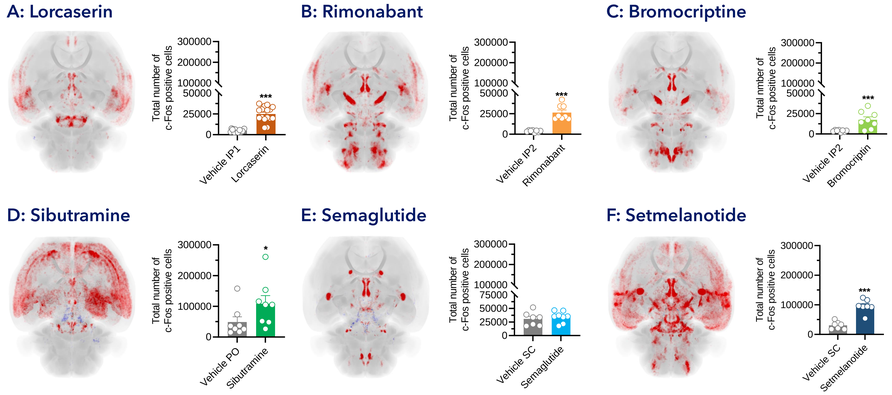
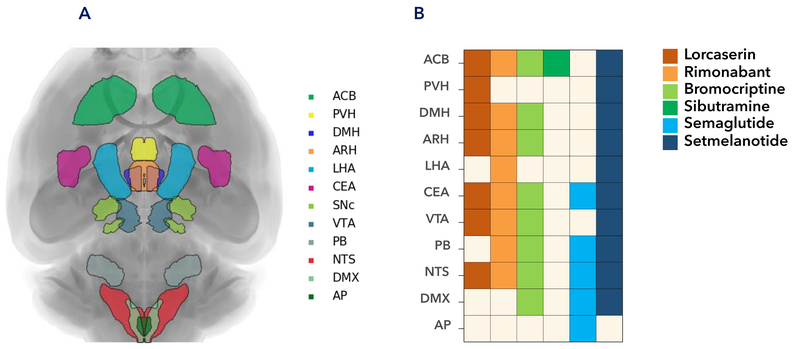



**Figure 2. 3D mapping and quantification of whole-brain c-Fos responses to acute treatment with various weight-loss promoting compounds.** The average c-Fos signal from n=8 mice mapped into the same 3D reference brain. Quantification and statistical analysis of c-Fos expression was performed in 308 brain regions. Brain areas with statistically significant changes in c-Fos expression ($p < 0.05$; Dunnett's test) are delineated in red (upregulation) or blue (downregulation) compared to corresponding vehicle controls. In general, the c-Fos response to peptide treatment (semaglutide), is more regioselective compared to that of small molecules and small peptides (e.g., lorcaserin, rimonabant, sibutramine, setmelanotide).

## 3 c-Fos expression signatures of weight-lowering drugs in major appetite-regulating brain regions




**Overlapping and specific c-Fos expression signatures of weight-lowering drugs in in key brain areas regulating energy homeostasis and hedonic eating. (A)** Anatomical map (dorsal view) depicting 12 selected brain regions involved in appetite regulation. **(B)** Summary of drug-induced c-Fos induction across the 12 individual brain regions. These 12 areas included cardinal hypothalamic feeding centres [paraventricular (PVH), dorsomedial (DMH) and arcuate (ARH) hypothalamic nucleus; lateral hypothalamic area, (LHA)]; central amygdalar nucleus (CEA); major dopaminergic pathways [nucleus accumbens (ACB), substantia nigra pars compacta (SNC), ventral tegmental area (VTA)] as well as components of the brainstem [parabrachial nucleus (PB), nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus nerve (DMX), area postrema (AP)]

## 4 Mapping quantitative changes in c-Fos expression

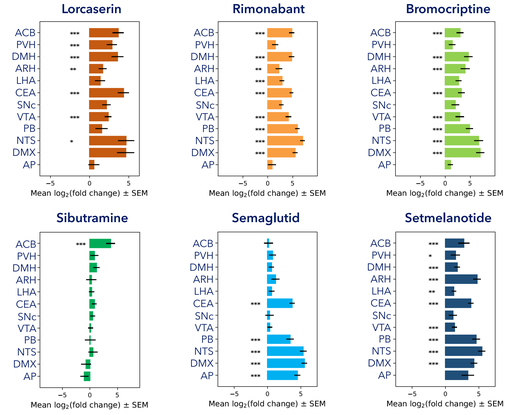



**Figure 4. Overlapping and distinct c-Fos expression signatures of weight-lowering drugs in major appetite-regulating brain regions.** Fold-change (log2 scale, mean ± S.E.M.) in c-Fos positive cell counts in the 12 selected brain regions (rostro-caudal order) compared to corresponding vehicle controls. Dunnett's test negative binomial generalised linear model with p-value adjustment for multiple comparisons using FDR < 0.05 was applied for statistical analysis (*p < 0.05; **p < 0.01, ***p < 0.001).

## Conclusion

+ We pinpoint several overlapping brain activation signatures of various weight-lowering drugs.
+ This shared feature suggests that weight-lowering drugs stimulate distinct homeostatic and non-homeostatic feeding centres.
+ Future centrally acting anti-obesity compounds may be specifically designed to target key components of this neurocircuitry framework to provide more effective and sustained weight loss in obese patients.

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