Whole-Brain 3D c-Fos Mapping Reveals Distinct Neuropsychiatric Drug Signatures

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Background & Aim

Psychiatric compounds modulate complex brain networks, making whole-brain assessment of neuronal activity essential for understanding their mechanisms of action. Activity-dependent genes such as c-Fos, and other stimulus-regulated genes (SRGs) provide complementary molecular readouts of neuronal activation, and transcriptional responses to treatment. Combined with 3D whole-brain imaging, these markers enable unbiased mapping of compound-specific neural circuit signatures, facilitating the discovery and characterization of novel therapeutics.

Methods

The mice were dosed with Emraclidine (orally, PO), xanomeline (Subcutenous, SC) or clozapine (PO). 2h after dosing, mice underwent perfusion with 10% neutral buffered formalin. Then, mouse brains were dissected, immunolabelled for c-Fos, cleared and imaged using light-sheet microscopy. The whole-brain c-Fos counts were quantitatively assessed using AI-assisted quantification. As a proof of concept, whole-brain ISH profiling of stimulus-responsive genes (SRGs) was performed using a similar 3D imaging pipeline following acute (2 h) and chronic (QD, 5 days) semaglutide treatment.

1 Schematic Overview of c-Fos Imaging Pipeline

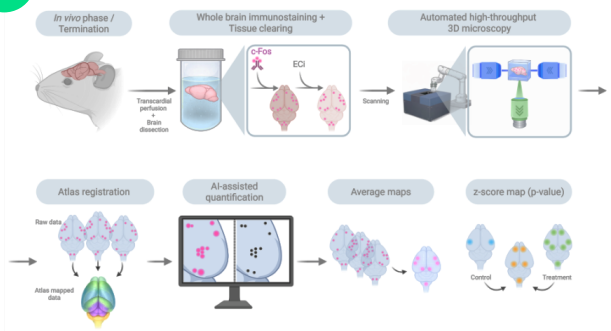


Figure 1. Whole brain staining, tissue clearing and AI image analysis schematic illustration.
Brains are immuno-stained for c-Fos, cleared, and imaged using light-sheet fluorescence microscopy. Then, brains are mapped to a reference atlas and the number of c-Fos positive cells is quantified using AI-assisted analysis. Figure created with BioRender.

2 c-Fos Up/Down Regulation Maps Identifies Distinct Patterns of Neuronal Engagement

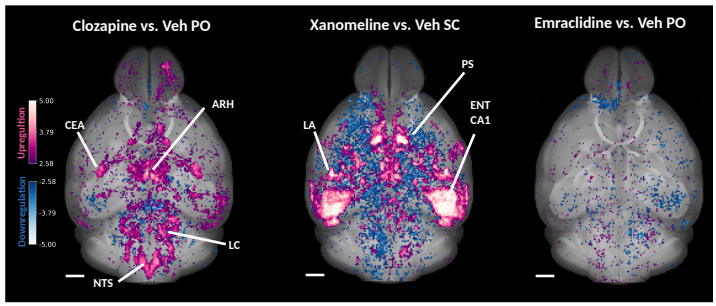


Figure 2. Comparison of whole brain c-Fos expression after dosing with different compounds. Overview of statistically significant differences in c-Fos counts across groups represented as Z-score maps. Areas displaying significant up- or downregulation are visualized in **hot** or **cold** colors, respectively. Z-score 2.58 corresponds to p<0.01 (FDR-uncorrected). Scale bar = 1000µm

3 Significantly Up and Down Regulated Brain Areas

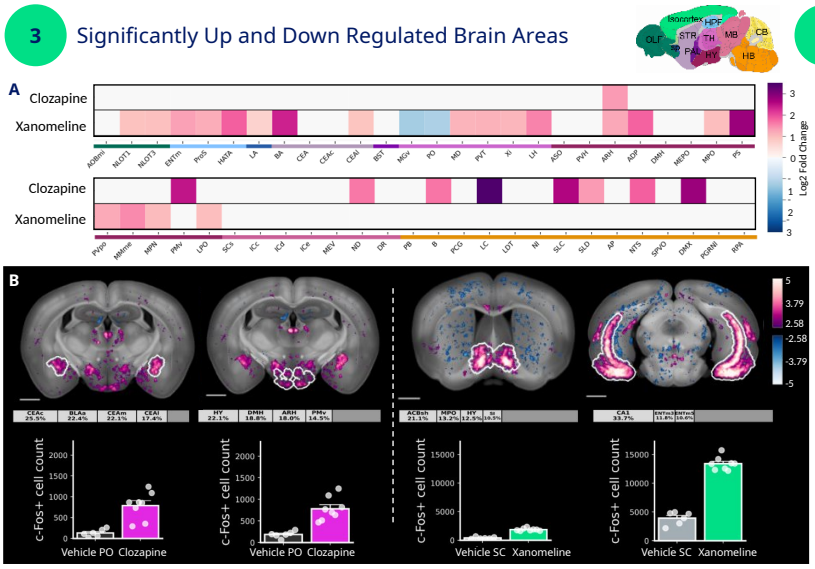


Figure 3. Top 5 Clusters of Compound-Induced c-Fos Modulation.
(A) Significant differences in c-Fos counts across groups. **Warm** colors indicate upregulation; **cool** colors indicate downregulation. **(B)** Example clusters of up or down-c-Fos regulation in Clozapine and Xanomeline vs. Vehicle. Brain area components of each cluster (in percentage) and relative c-Fos cell counts. Scale bar = 1000µm.

4 Comparative Analysis of Compound Similarity Profiles

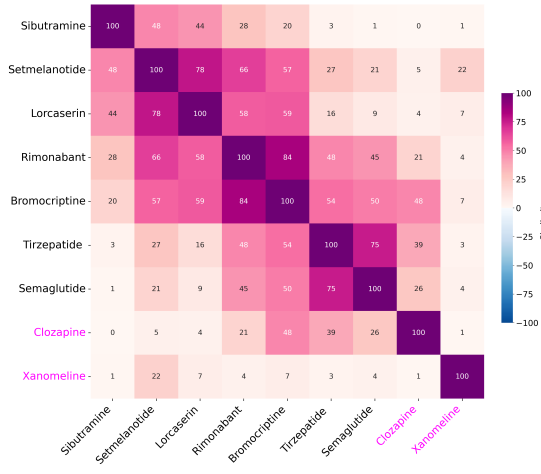
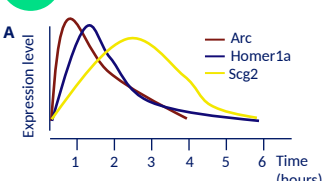


Figure 4. Compound similarity index of Test compounds vs. Reference compounds. Test compounds are compared among themselves and to Reference compounds. Whole-brain c-Fos counts are used to compute a similarity index (0-100). Compounds are sorted using hierarchical clustering. All group-comparisons with no significant regions are not included in this plot.

5 Profiling of Activity Dependent Response Genes Using Whole-Brain ISH



Rapid PRGs: cFos, Npas4, Nr4a1, Arc, Erg1
Delayed PRGs: Crem, BDNF
SRGs: Homer1a, Scg2, Nptx2

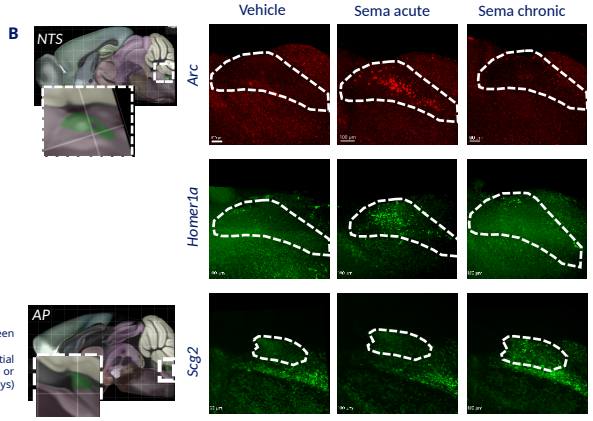


Figure 5. RNA Profiling of Activity Dependent Response Genes
(A) Schematic timeline illustrating the temporal relationship between activity-dependent gene expression following treatment or stimulation.
(B) Representative high-resolution 3D imaging images showing the spatial expression of different probes within the nucleus tractus solitarius (NTS) or area postrema (AP) following acute (2 hours) and chronic (QD, 5 days) Semaglutide treatment. Scale bar = 100µm.

Conclusion

+ High-throughput whole-brain 3D c-Fos mapping identified distinct neuronal activation signatures for Clozapine and Xanomeline, revealing compound-specific patterns of activity across key brain circuits involved in psychiatric disorders.
+ Integration of whole-brain imaging with ISH profiling of stimulus-responsive genes (SRGs) enabled the temporal characterization of activity-dependent molecular responses following both acute and chronic treatment.
+ This multimodal pipeline provides a unique and powerful approach for neuropsychiatric drug characterization, combining whole-brain cellular-resolution imaging with temporal molecular profiling, and high-throughput scalability.

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