Molecular Abnormalities in Schizophrenia: Neurotransmission, Oxidative Stress, and Apoptosis

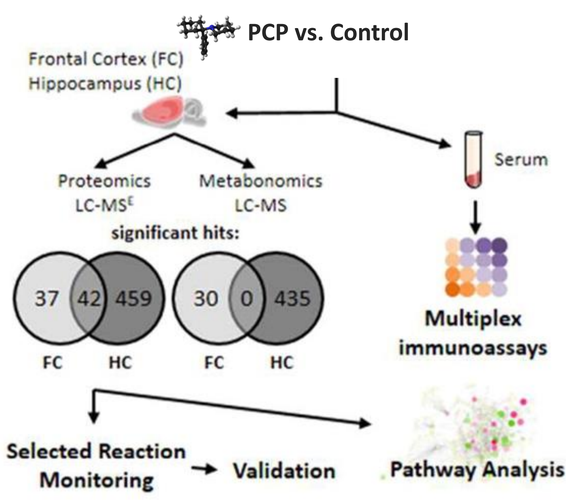
Elizabeth J. Want,[1] Hendrik Wesseling,[2] Elaine Holmes,[1] and Sabine Bahn[2]

[1]Section of Biomolecular Medicine, Division of Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College, London SW7 2AZ, U.K.
[2]Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB2 1QT, U.K.

Background

- Schizophrenia affects 1% of the world's population.
- Broad, heterogeneous range of symptoms hinders the effectiveness of current therapies.
- New models reproducing core schizophrenia features are needed to **elucidate pathological disease mechanisms, identify biomarkers for improved diagnosis and discover potential novel drug targets**.
- A multi-platform approach was employed to analyse distinct brain regions implicated in schizophrenia.

Figure 1. Workflow overview.



Methods I: Metabolic profiling

- Brain tissue samples extracted using optimised in-house protocols.
- Quality control (QC) samples produced by combining 10 µL aliquot from each study sample.
- UPLC–MS analysis using a Waters XEVO G2 Q-TOF mass spectrometer coupled to an Acquity UPLC system.
- Separation on a 2.1 × 100 mm (1.7 µm) HSS T3 Acquity column with a 28 min linear gradient of water: methanol.
- Data processed using the freeware XCMS with standard parameters.
- Metabolite tables (*m/z*, RT, intensity) imported into SIMCA-P (Umetrics) for multivariate analysis.

Methods II: Label-Free LC–MSE Analysis

- Digested brain tissue samples analysed using a nanoACQUITY coupled to a Waters Q-TOF Premier mass spectrometer.
- Fragment ions matched to corresponding precursor peptide ions using RT, mass accuracy.
- Data processed with ProteinLynx Global Server v.2.4 (Waters) and Rosetta Elucidator v.3.3 (Rosetta).
- Aligned peaks extracted and abundance measurements obtained by integrating retention time, *m/z*, and intensity values, with normalization to total ion current.
- PLGS2.4 using the Swiss-Prot rodent reference proteome used for protein identification searches.

Methods III: *Label-Based Selected Reaction Monitoring (SRM) MS*

- Digested frontal cortex and hippocampus proteomes analyzed using targeted label-based SRM MS on a Xevo TQ-S mass spectrometer coupled to a nanoAcquity UPLC system (Waters Corporation; Milford, MA, USA).
- Multiplex SRM assays developed using a high throughput strategy. Resulting SRM data analysed using Skyline.
- Differential abundance of analytes between cPCP-treated rats and control animals calculated using MSstats.

Results I: *Metabolic profiling*

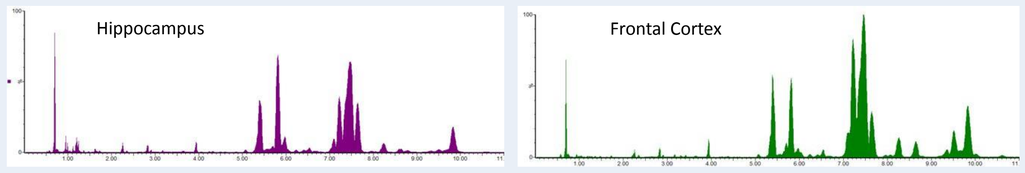


Figure 2. UPLC-MS BPI Chromatograms of brain tissue extracts (Lipid profiling, ESI+ mode).

- cPCP showed a greater effect in the **hippocampus**.
- 1057 metabolite features identified across both models and brain regions.
- No significantly changed features were detected in the frontal cortex.
- **426 metabolite features significantly changed in the hippocampus.**
- Metabolite ID on top 10 significant hits using HMDB and Pubchem databases.
- Main metabolic changes were observed in **glycerophospholipids;** specifically ***phosphatidylcholines, phosphatidylserines, phosphatidylglycerol*** and ***glycerophosphoglycerophosphates*.**

Results II: *Proteomics*

- IL-5, IL-2, IL-1β, fibroblast growth factor-2 (FGF-2),macrophage inflammatory protein 1a (MIP-1α) significantly altered in cPCP serum
- 555 proteins identified in the frontal cortex. **79 proteins** (14%) significantly changed due to cPCP treatment.
- 937 proteins identified in the hippocampus. **501 proteins** (53%) significantly changed due to cPCP treatment.
- Protein level alterations of 22 enzymes detected in the frontal cortex, of which 10 (45%) catalyse a metabolic reaction
- Protein level alterations of 139 enzymes detected in the hippocampus, of which 94 (68%) catalyse a metabolic reaction.

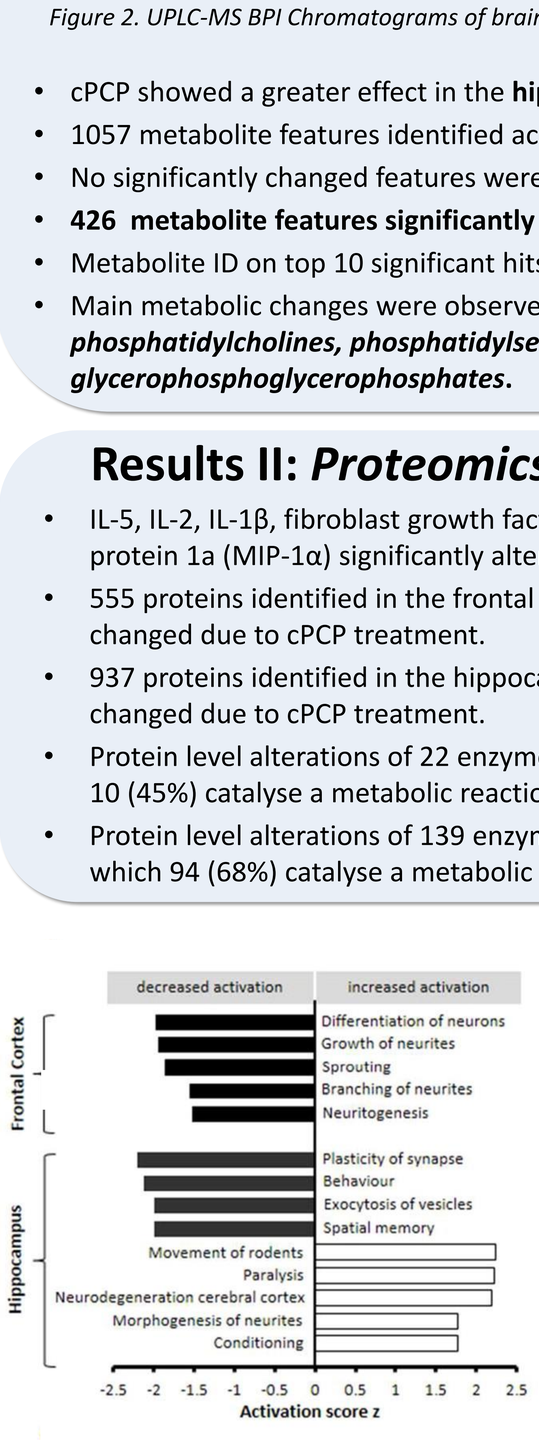


Figure 4. Computational pathway analysis of cPCP-induced proteomic alterations in frontal cortex and hippocampus. Ingenuity Pathway Analysis (IPA) showing significantly ↓ and ↑ biological functions in cPCP rat brain regions. Functions ($p < 0.05$) shown with activation score (z-score) >1 (increased activation) or <−1 (decreased activation).

Pathway Analysis

- Ingenuity Pathways Analysis (IPA): ↓ **in neurodevelopment associated biological functions in frontal cortex**.
- Hippocampus: ↓ activation of biological processes, plasticity of synapse, exocytosis of vesicles, behaviour, spatial memory, and ↑ activation of movement of rodents, paralysis and conditioning.
- Matches the reported behavioural readouts associated with the cPCP model in the literature.
- GO-enrichment analysis of proteomic changes revealed the most robust enriched biological functions across both brain regions associated with small GTPases and Rho signalling proteins.

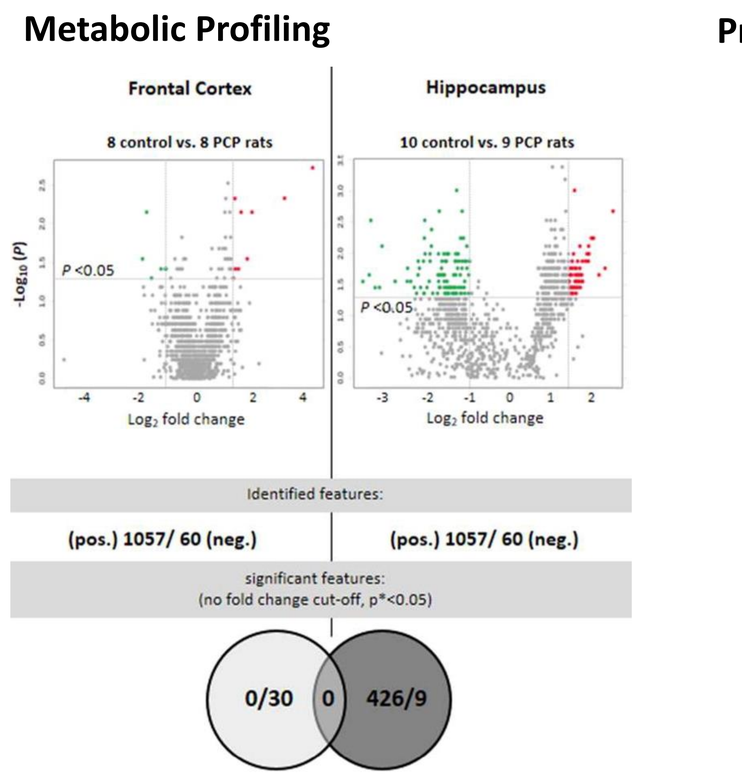


Figure 3. Volcano plots of group comparisons showing adjusted significance values versus fold-change distributions. Horizontal grey lines = adjusted p-value threshold of 0.05, vertical grey dotted lines = fold-change threshold of 10% (proteomics) and 50% (metabonomics, ESI+ mode). Green dots = down-regulated proteins & metabolites, red dots = up-regulated proteins & metabolites. Grey and black dots = proteins & metabolites not meeting threshold.

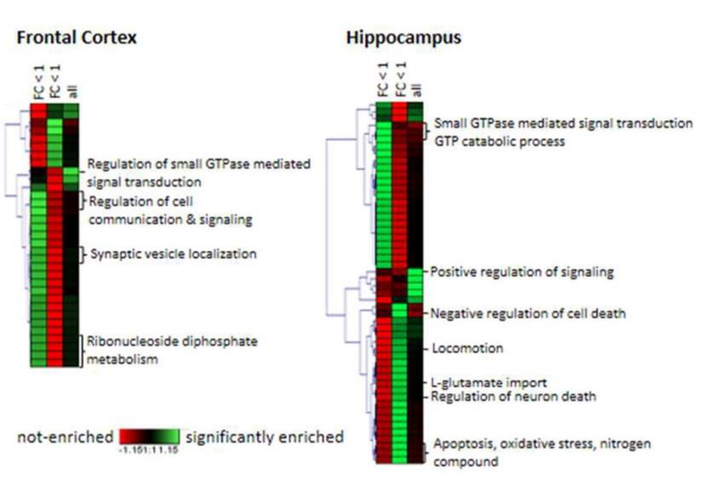


Figure 5. Functional enrichment analysis of significantly changed proteins in the frontal cortex and hippocampus of the cPCP rat. Proteins split into fold-change bins for analyses. Colour-coded z-score-transformed p-values indicate the significance of the enrichment for each bin as indicated.

Conclusions

- cPCP treatment induced protein changes were subtle, indicating homeostatic disequilibrium.
- Larger effect on hippocampal proteome and metabonome compared to frontal cortex.
- Trend toward an anti-inflammatory state, with alterations in cytokine levels (IL-5, IL-2, IL-1β) and fibroblast growth factor-2.
- Changes in lipid metabolism, particularly glycerophospholipids, supported through altered superoxide dismutase levels, indicative of oxidative stress and apoptotic pathway alterations.
- Abnormalities in NMDA-receptor associated pathways in both brain regions
- Alterations in kainate, AMPA and GABAergic signalling in the hippocampus.
- Findings correlated with hippocampal behavioural functions.
- Molecular changes parallel human findings. Could lead to increased understanding of how perturbed glutamate receptor signalling affects other biological pathways in schizophrenia.
- May lead to discovery of novel drug targets for improved treatment; surrogate markers in blood can be translated to the clinic.

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