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Effect of an anti-depressant on mouse hippocampus protein turnover using MIMS

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Abstract

Although antidepressants have been used in the treatment of affective disorders for over fifty years, the precise mechanism of their action remains unknown. Treatment regimens are based by and large on empirical parameters and characterized by a trial and error scheme. A better understanding of the mechanisms involved in antidepressant drug response is of fundamental importance for the development of new compounds that have a higher success rate and specificity. In order to elucidate the molecular pathways involved in the action of antidepressants, we wish to identify brain areas, cell types, and organelles that are targeted by antidepressant treatment in mice. Multi-isotope Imaging Mass Spectrometry (MIMS) allows a quantitative approach to this analysis, allowing us to delineate antidepressant effect on protein synthesis in the brain at single cell and organelle resolution. In these experiments, we obtained a global analysis of protein turnover in the hippocampus *dentate gyrus* (DG) and in the *Cornu Ammonis* (CA) regions, together with a subcellular analysis in the granular cells and others.

Keywords

Antidepressant; MIMS; Multi-isotope Imaging Mass Spectrometry; Protein turnover; Hippocampus

Introduction

Affective disorders, including depression, are major public health problems and have become a predominant cause for disability in the Western world. Pharmacotherapy of depression relies almost entirely upon drugs that modulate monoaminergic neurotransmission in the brain, a pharmacological principle discovered serendipitously over fifty years ago. Though major progress has been made regarding the adverse effects of these drugs, their efficacy continues to be dissatisfying. Treatment regimens are based by and large on empirical parameters and characterized by a trial and error scheme. Therapy with the antidepressants that are available at the present time requires long-term treatment. Furthermore, the rates of remission (i.e., absence of depressive symptoms) remain low

(20-30%) after five to six weeks of treatment and the response rates (i.e., improvement by about 50% from baseline) after the same treatment period are on average no better than 60-70%. A better understanding of the mechanisms involved in antidepressant drug response is of fundamental importance for the development of new compounds that have a higher success rate and specificity. In order to elucidate the molecular pathways involved in the action of antidepressants, we wish to identify brain areas, cell types, and organelles that show a change in protein turnover in response to antidepressant treatment in inbred mouse strains.

The *Proteomics and Biomarkers* research group at the *Max Planck Institute of Psychiatry* has focused its biomarker discovery efforts on the analysis of mouse models for affective and neurological disorders.^{1, 2, 3} Metabolic labeling of mouse models with stable isotopes^{4, 5} has allowed us to analyze protein expression differences of a quantitative as well as qualitative nature by comparative mass spectrometry.^{3, 6} We have successfully optimized the metabolic labeling procedure in mouse models of trait anxiety by using a novel ¹⁵N-labeled mouse diet based on *Ralstonia eutropha* biomass that was co-developed with *Silantes GmbH*.⁵

Method

Mice were partially metabolically labeled with a ¹⁵N diet, mouse brains sectioned and then processed for either MIMS or tandem mass spectrometry proteome analyses. For MIMS analysis, 12 DBA/20la mice were fed with ¹⁵N enriched diet (spirulina, ¹⁵N, 98%+) for 7 days, metabolically labeling the entire animal proteome with ¹⁵N (Figure 1). Six mice were treated with paroxetine and 6 mice with control vehicle. The dosage was 10mg paroxetine / kg body weight, administered orally via tap water or by gavage. There was no chase period; animals were sacrificed on Day 7.

The hippocampus was dissected, fixed in osmium solution, and embedded. Thin sections were analyzed with MIMS.^{8, 9} Mosaics composed of hundreds of individual MIMS images (tiles) were created using OpenMIMS (Image J plug-in) and Utah Nrrd Utilities (unu). This generates many adjacent images on a tissue section and allows us to analyze areas of over 1 mm² at a resolution on the order of 100 nm (Figures 2 and 3).

Results

The mosaics allowed us to obtain a global analysis of protein turnover in the hippocampus *dentate gyrus* (DG) and in the *Cornu Ammonis* (CA) regions, together with a subcellular analysis in the granular cells and others. Overall, after 1 week of labeling without paroxetine the lowest level of turnover was found in the DG neurons and the highest in the CA3 cells. Paroxetine treatment did not effect protein turnover in the DG, increased protein turnover in nucleus and cytoplasm in CA3 and decreased protein turnover in nucleus and cytoplasm in CA4. (Table 1)

Discussion and Conclusion

Protein turnover alterations in subcellular compartments are region-specific. Treatment affects protein turnover in a region- and subcellular compartment- specific manner. Paroxetine's mode of action and hippocampal neurogenesis in DG may be related with the protein turnover differences observed here.

MIMS technology facilitates analyses that cannot be achieved with any other brain imaging technology: delineation of antidepressant effect on protein synthesis in the brain at single cell and organelle resolution. This will help identify pathways pertinent to the antidepressant drug response. Support Vector Machine segmentation¹⁰ (Figure 4) will greatly help to reduce the immense amount of data.

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References

1. Krömer SA, Kessler MS, Milfay D, Birg IN, Bunck M, Czibere L, Panhuysen M, Pütz B, Deussing JM, Holsboer F, Landgraf R, Turck CW. *J Neurosci*. 2005; 25(17):4375–4384. [PubMed: 15858064]
2. Ditzgen C, Varadarajulu J, Czibere L, Gonik M, Targosz BS, Hambsch B, Bettecken T, Kessler MS, Frank E, Bunck M, Teplytska L, Erhardt A, Holsboer F, Mller-Myhsok B, Landgraf R, Turck CW. *Mol Psychiatry*. 2010; 15(7):702–711. [PubMed: 19139748]
3. Filiou MD, Zhang Y, Teplytska L, Reckow S, Gormanns P, Maccarrone G, Frank E, Kessler MS, Hambsch B, Nussbaumer M, Bunck M, Ludwig T, Yassouridis A, Holsboer F, Landgraf R, Turck CW. *Biol Psychiatry*. 2011; 70(11):1074–1082. [PubMed: 21791337]
4. Wu, CC.; MacCoss, MJ.; Howell, KE.; Matthews, DE.; Yates, JR, 3rd. *Anal Chem*. Vol. 76. Washington, DC, U S: 2004. p. 4951-4959.
5. Frank E, Kessler MS, Filiou MD, Zhang Y, Maccarrone G, Reckow S, Bunck M, Heumann H, Turck CW, Landgraf R, Hambsch B. *PLoS One*. 2009; 4(11):e7821. [PubMed: 19915716]
6. Zhang Y, Filiou MD, Reckow S, Gormanns P, Maccarrone G, Kessler MS, Frank E, Hambsch B, Holsboer F, Landgraf R, Turck CW. *Mol Cell Proteomics*. 2011; 10(12):M111.008110.
7. Webhofer C, Zhang Y, Brusis J, Reckow S, Landgraf R, Maccarrone G, Turck CW, Filiou MD. *J Proteomics*. 2013; 88:27–33.10.1016/j.jprot.2012.12.013 [PubMed: 23279933]
8. Lechene C, Hillion F, McMahon G, Benson D, Kleinfeld AM, Kampf P, Distel D, Luyten Y, Bonventre J, Hentschel D, Park KM, Ito S, Schwartz M, Benichou G, Slodzian G. *J Biol*. 2006; 5(6):20. [PubMed: 17010211]
9. Zhang DS, Piazza V, Perrin BJ, Rzdazinska AK, Poczatek JC, Wang M, Prosser HM, Ervasti JM, Corey DP, Lechene CP. *Nature*. 2012; 481(7382):520–524. [PubMed: 22246323]
10. Gormanns P, Reckow S, Poczatek JC, Turck CW, Lechene C. *PloS One*. 2012; 7:e30576. [PubMed: 22347386]

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1

Percent Protein Turnover

	DG						CA3						CA4					
	Vehicle			Drug			Vehicle			Drug			Vehicle			Drug		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Cytoplasm	470	29.7	4.6	410	30.3	4.3	140	32.1	4.1	210	34.9	5.7	406	34.4	5.3	246	30.5	4.2
Nucleus	2350	25.6	4.6	2050	26.0	3.7	200	35.2	4.7	300	39.1	7.0	580	35.1	6.1	350	31.3	4.7

DG = dentate gyrus, CA3 = Cornu Ammonis region 3, CA4 = Cornu Ammonis region 4, N = number of regions of interest (ROIs) drawn on the quantitative images from which data was extracted, Mean = mean of ROIs for a given group, SD = standard deviation.