Dataset Brief:

Proteome and pathway effects of chronic haloperidol treatment in mouse hippocampus

Authors:

Klaus Oliver Schubert [1,2]

Melanie Föcking [1]

Kieran Wynne[3]

David R. Cotter[1]

[1]Department of Psychiatry, Royal College of Surgeons in Ireland, Dublin, Ireland
[2]Discipline of Psychiatry, The University of Adelaide, Adelaide, Australia
[3]Proteomics Resource, UCD Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland

K.O.S and M.F. contributed equally to this work, and should both be considered first authors

Abstract

Proteomic exploration of the effects of psychotropic drugs on specific brain areas in rodents has the potential to uncover novel molecular networks and pathways affected by psychotropic medications, and may inform etiologic hypotheses on mental disorders, aiding drug discovery and improved side effect management. Haloperidol is a widely used first-generation antipsychotic. Treatment with haloperidol has been shown to produce structural and functional changes of the hippocampus. At the same time, the hippocampus has been implicated in the neuropathology of psychotic disorders such as schizophrenia and bipolar disorder.

Seven male C57BL/6 mice, at 10 weeks of age, were injected daily intraperitoneally with 0.5 mg/kg of haloperidol, for 28 days. A control group of six animals was injected with vehicle only (saline). Animals were sacrificed, and protein levels of hippocampus homogenate were determined using a label-free liquid chromatography/tandem mass spectrometry (LC/MS/MS). Pathway analysis of proteins identified as differentially expressed in haloperidol-treated mice was undertaken using Ingenuity Pathway Analysis (IPA). Statistical testing of mean differences of Label-Free-Quantification (LFQ)-intensities in haloperidol-treated mice versus controls, identified 216 differentially expressed proteins with p-values <0.05. IPA analysis implicated oxidative phosphorylation and mitochondrial function as top canonical pathways. Identified proteins are overrepresented in networks involved in tubulin-mediated cytoskeleton dynamics, clathrin-mediated endocytosis, and extracellular signal-regulated kinase (ERK) - and c-Jun N-terminal kinase (JNK) signaling.

The findings of this study have the potential to stimulate further research into protein networks, biological pathways, and cellular mechanisms associated with haloperidol treatment. Findings may generate testable hypothesis for the exploration of predictive biomarkers for haloperidol treatment response and side effects. The MS data have been deposited with the ProteomeXchange Consortium with dataset identifier …

There is accumulating evidence that long-term exposure of the human and animal brain to the widely used butyrophenone antipsychotic haloperidol, prescribed for the treatment of schizophrenia and other psychotic disorders [1], can result in structural and functional changes, such as decreasing grey matter volumes [2-4], altered brain neuroreceptor expression [5, 6], decreases in resting cerebral blood flow [7, 8], and compromised cellular morphology [9-12]. The hippocampus, a temporal lobe structure involved in memory consolidation, appears particularly sensitive to the exposure to antipsychotic medications including haloperidol in humans and rodents [13-19]. In rats, chronic exposure to haloperidol was shown to increase hippocampal volume [20] in the contrast to prominent volume loss in other brain regions such as the anterior cingulated cortex (ACC) [21, 22]. Currently, it is unclear which molecular signaling mechanisms drive structural and functional brain changes, and whether these mechanisms contribute to the therapeutic effects of antipsychotic drugs or represent neurodegenerative effects independent of the disorders that they are intended to treat [23-27].

Proteomic investigations, examining brain tissue in animal models, offer an opportunity for the generation of testable hypotheses about the molecular mechanisms affected by exposure to antipsychotics such as haloperidol. Proteomic findings could lead to improved understanding of the therapeutic and adverse effects of these widely used medications. Therefore, the aim of the current study was to investigate the proteomic effects of long-term haloperidol treatment on the hippocampus of C57/BL6 mice.

Ethical approval for these experiments was granted by the RCSI Research Ethics Committee (application no. REC175). Seven male C57BL/6 mice, at 10 weeks of age, were injected daily intraperitoneally with 0.5 mg/kg of haloperidol, for 28 days. A control group of or six animals was injected with vehicle only (saline). Immediately after decapitation, the hippocampus was dissected, snap frozen in liquid nitrogen, and stored at -80°C. Time frame of treatment and intraperitoneal dose were determined in accordance to previously published literature, suggesting that 28 days of daily antipsychotic exposure in mice models chronic drug treatment in humans [28-30].

Samples were prepared for Liquid Chromotography/ tandem mass spectrometry (LC/MS/MS) as described previously in detail [31]. In brief, hippocampi were homogenized by sonication, and protein concentration of each homogenate was determined using the Bradford assay [32]. Homogenates were subsequently denatured, reduced, and alkylated before tryptic digestion. Peptides were then resuspended in 0.5% TFA, dried, and desalted using Zip-Tips (Millipore, USA). Label-free LC/MS/MS was conducted using a Thermo Scientific LTQ ORBITRAP XL mass spectrometer connected to a Dionex Ultimate 3000 (RSLCnano) chromatography system. Tryptic peptides were resuspended in 0.1% formic acid. Each sample was loaded onto Biobasic Picotip Emitter (120 mm length, 75 μm ID) packed with Reprocil Pur C18 (1.9 μm) reverse phase media column, and was separated by an increasing acetonitrile gradient, using a 120 min reverse phase gradient at a flow rate of 250 nL/min. The mass spectrometer was operated in positive ion mode with a capillary temperature of 200 °C, a capillary voltage of 45V, a tube lens voltage of 100V, and with a potential of 1800V applied to the frit. All data

were acquired with the mass spectrometer operating in automatic data-dependent switching mode. A high-resolution MS scan (300-2000 Dalton) was performed using the Orbitrap to select the 7 most intense ions prior to MS/MS analysis using the Ion trap.

Statistical analysis of Orbitrap data was carried out using Maxquant analysis software (Max Planck Institute of Biochemistry, Martinsried, Germany). Results were searched against rodent databases.

Pathway analysis was carried out using the proprietary Ingenuity Pathway Analysis Software (IPA, Quiagen, Redwood City, USA, http://www.ingenuity.com).

In total, 1068 distinct proteins of the mouse hippocampus were identified using LC/MS/MS technology. Statistical testing of mean differences of Label-Free-Quantification (LFQ)-intensities in haloperidol-treated mice versus controls, identified 216 proteins with p-values <0.05 [range p=0.0001 to p=0.049] (Supplementary Table 1).

False Discovery Rate (FDR)-adjusted p-values (q-values) for these tests ranged between q=0.09 and q=0.24. Seven proteins with q<0.1, 60 proteins with q<0.13, and 67 proteins with q<0.15 were identified. Corresponding fold changes were calculated, ranging from 6.2-fold reduced expression of Apolipoprotein A-1 binding protein (Apoa1bp), to a 3.7-fold over-expression of Gamma actin-like protein (Actg1) in haloperidol-treated mice, as compared to controls (Table S1). 74 of the 216 differentially expressed proteins (34%) had fold changes > ± 1.5.

IPA analysis was carried out on all 216 proteins demonstrating mean expression differences associated with p-values < 0.05. The IPA Associated Network Functions tool identified 5 top protein networks affected by chronic haloperidol treatment in mouse hippocampus (Table 1). A graphic representation of the top protein networks 1 is depicted in Figure 1. Graphs illustrating networks 2 and 3 are shown in the supplementary material (Supplementary Figure 1, and Supplementary Figure 2). Networks include proteins of the original dataset, as well as non-dataset proteins that are associated via network functions.

[Insert Table 1 about here]

[Insert Figure 1 about here]

The top 5 IPA canonical pathways affected by chronic treatment with haloperidol were oxidative phosphorylation (13 proteins), mitochondrial dysfunction (13 proteins) epithelial adherens junction signaling (11 proteins), signaling by rho family GTPases (11 proteins), and germ cell – sertoli cell junction signaling (9 proteins) (Table 2).

Hippocampal proteins affected by chronic haloperidol administration have previously been implicated in neurological disease, psychological disorders, skeletal and muscular disorders, hereditary disorders, and cancer, according to the IPA Diseases and Disorders database (Supplementary Table 2). In terms of IPA-defined cellular functions affected by haloperidol exposure, nucleic acid

metabolism, small molecule biochemistry, cell death and survival, energy production, and cell cycle were most predominantly implicated (Supplementary Table 3). In addition, IPA reported the top toxicology pathways altered as a consequence of haloperidol treatment (Supplementary Table 4). These were mitochondrial dysfunction (13 proteins), oxidative stress (4 proteins), fatty acid metabolism (5 proteins), oxidative stress (4 proteins), cholesterol biosynthesis (2 proteins), and renal necrosis/cell death (10 proteins).

[Insert Table 2 about here]

The results of this study are of potential interest in interpreting molecular effects of haloperidol on the hippocampus. For example, the central node proteins of IPA protein network 1 (Fig 1), tubulin and Cullin-associated Nedd8-dissociated protein 1 (CAND1), have previously been implicated in antipsychotic drug action. Cellular tubulin, which was overexpressed in our study in haloperidol-treated mice (Supplentary Table 1) forms cytoskeletal microtubules and is involved in many central cellular processes, including cell division and cellular transport of secretory vesicles, organelles, and intracellular substances [33]. Disrupted microtubule function has been implicated in neuropsychiatric disorders such as schizophrenia and bipolar disorder on the basis of morphological and molecular findings [34-41], and previous proteomic investigations have found strong associations between psychotic disorders and the tubulin-associated cytoskeleton [42]. Recent evidence suggests that pharmacological stabilization of microtubules could constitute an important molecular mechanism of antipsychotic and mood-stabilizing medications [43],

which appears to be closely linked to the intracellular transport of mitochondria and the associated energy supply to existing or forming synapses [44].

CAND1, which we found overexpressed in this study (Supplementary Table 1), is a regulator protein of Cullin-RING ubiquitin (Ub) ligases (CRLs) that are responsible for ubiquitinylation of approximately 20% of all proteins degraded by the Ub proteasome system (UPS) [45]. A recent study showed that increases of CAND1 protein induces adipogenensis from preadipocytes via regulation of the the cyclin-dependent kinase (CDK) inhibitor p27 [46]. In the context of haloperidol treatment, up-regulating effects on CAND1 signaling may be one explanation for the effects of haloperidol and other antipsychotics on weight gain and metabolic syndrome in treated patients.

Effects of haloperidol on mitochondrial function, the electron transport chain, and oxidative phosphorylation have been documented extensively by previous studies, and IPA identified oxidative phosphorylation and mitochondrial function as top 3 canonical pathways affected by the drug in mouse hippocampus. Previously, the inhibition of electron transfer activity at respiratory complex I, and concomitant increases complex IV activity by haloperidol have been reported in rodent experiments [47, 48]. Overall, these effects likely result in the inhibition of the maximal oxygen consumption rate after mitochondrial uncoupling, and reduced cellular ability to produce ATP [48]. The findings suggest that antipsychotics such as haloperidol might contribute to compromised bioenergetic function, which in turn may be involved in the cellular pathology underlying severe haloperidol side effects such as tardive dyskinesia (TD). On the other hand, anti-mitochondrial effects may also play a part in haloperidol's antipsychotic action.

This study has a number of limitations. Firstly, results were no longer statistically significant at a 5% threshold after adjusting for False Discovery Rate (FDR), which was applied to control for multiple testing. Proteomic findings across many fields of research have traditionally struggled to hold up to FDR, due to the significant variability measured across many experiments. Additionally, the statistical specifics of applying FDR to large proteomic datasets have remained somewhat controversial, implying that its use may result in overly stringent discarding of valuable data [49]. In our view, the global protein changes identified on the p-value level in the analysis, remain valuable for pointing to underlying biological pathways and mechanisms if applied to pathway analysis software such as IPA. Additionally, if the results serve to follow up on specific hypotheses concerning only small groups of candidate proteins, FDR becomes obsolete. Secondly, for the present investigation, only adult mice were used. It is possible that the same investigation in adolescent mice might have resulted in different findings. Studies on the effects of antipsychotics and other psychotropic drugs show differential effects in young and adult rats [50, 51]. Thirdly, the findings of this LC/MS/MS study have not been confirmed using alternative techniques. Confirmation experiments using Western blotting or ELISA would be paramount before specific candidates are taken forward for further analysis.

In summary, the present study has used a high-throughput proteomic platform to achieve in-depth coverage of the proteome of the adult mouse hippocampus following long-term administration of haloperidol. These data contribute to the understanding of the mechanistic action of haloperidol in rodent brain, and

possibly point to mechanisms in humans underlying therapeutic and adverse effects.

References

[1] Leucht, S., Cipriani, A., Spineli, L., Mavridis, D.*, et al.*, Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet* 2013, *382*, 951-962.

[2] Gur, R. E., Maany, V., Mozley, P. D., Swanson, C.*, et al.*, Subcortical MRI volumes in neuroleptic-naive and treated patients with schizophrenia. *The American journal of psychiatry* 1998, *155*, 1711-1717.

[3] Lieberman, J. A., Tollefson, G. D., Charles, C., Zipursky, R.*, et al.*, Antipsychotic drug effects on brain morphology in first-episode psychosis. *Archives of general psychiatry* 2005, *62*, 361-370.

[4] Ho, B. C., Andreasen, N. C., Ziebell, S., Pierson, R., Magnotta, V., Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. *Archives of general psychiatry* 2011, *68*, 128-137.

[5] Mackay, A. V., Iversen, L. L., Rossor, M., Spokes, E.*, et al.*, Increased brain dopamine and dopamine receptors in schizophrenia. *Archives of general psychiatry* 1982, *39*, 991-997.

[6] Kornhuber, J., Riederer, P., Reynolds, G. P., Beckmann, H.*, et al.*, 3H-spiperone binding sites in post-mortem brains from schizophrenic patients: relationship to

neuroleptic drug treatment, abnormal movements, and positive symptoms. *Journal of neural transmission* 1989, *75*, 1-10.

[7] Lahti, A. C., Weiler, M. A., Holcomb, H. H., Tamminga, C. A., Cropsey, K. L., Modulation of limbic circuitry predicts treatment response to antipsychotic medication: a functional imaging study in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2009, *34*, 2675-2690.

[8] Snitz, B. E., MacDonald, A., 3rd, Cohen, J. D., Cho, R. Y.*, et al.*, Lateral and medial hypofrontality in first-episode schizophrenia: functional activity in a medication-naive state and effects of short-term atypical antipsychotic treatment. *The American journal of psychiatry* 2005, *162*, 2322-2329.

[9] Lidow, M. S., Song, Z. M., Castner, S. A., Allen, P. B.*, et al.*, Antipsychotic treatment induces alterations in dendrite- and spine-associated proteins in dopamine-rich areas of the primate cerebral cortex. *Biological psychiatry* 2001, *49*, 1-12.

[10] Selemon, L. D., Lidow, M. S., Goldman-Rakic, P. S., Increased volume and glial density in primate prefrontal cortex associated with chronic antipsychotic drug exposure. *Biological psychiatry* 1999, *46*, 161-172.

[11] Konradi, C., Heckers, S., Antipsychotic drugs and neuroplasticity: insights into the treatment and neurobiology of schizophrenia. *Biological psychiatry* 2001, *50*, 729-742.

[12] Altunkaynak, B. Z., Ozbek, E., Unal, B., Aydin, N.*, et al.*, Chronic treatment of haloperidol induces pathological changes in striatal neurons of guinea pigs: a light and electron microscopical study. *Drug and chemical toxicology* 2012, *35*, 406-411.

[13] Mamah, D., Harms, M. P., Barch, D., Styner, M.*, et al.*, Hippocampal shape and volume changes with antipsychotics in early stage psychotic illness. *Frontiers in psychiatry* 2012, *3*, 96.

[14] Pizzolato, G., Soncrant, T. T., Rapoport, S. I., Haloperidol and cerebral metabolism in the conscious rat: relation to pharmacokinetics. *Journal of neurochemistry* 1984, *43*, 724-732.

[15] Wotanis, J., Hanak, S. E., Wettstein, J. G., Black, M. D., Comparative analysis of acute and chronic administration of haloperidol and clozapine using [3H] 2-deoxyglucose metabolic mapping. *Schizophrenia research* 2003, *61*, 195-205.

[16] Miller, D. D., Andreasen, N. C., O'Leary, D. S., Rezai, K.*, et al.*, Effect of antipsychotics on regional cerebral blood flow measured with positron emission tomography. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 1997, *17*, 230-240.

[17] Cohen, R. M., Nordahl, T. E., Semple, W. E., Pickar, D., The brain metabolic patterns of clozapine- and fluphenazine-treated female patients with schizophrenia: evidence of a sex effect. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 1999, *21*, 632-640.

[18] Molina, V., Gispert, J. D., Reig, S., Sanz, J.*, et al.*, Cerebral metabolism and risperidone treatment in schizophrenia. *Schizophrenia research* 2003, *60*, 1-7.

[19] Lahti, A. C., Holcomb, H. H., Weiler, M. A., Medoff, D. R.*, et al.*, Clozapine but not haloperidol Re-establishes normal task-activated rCBF patterns in schizophrenia within the anterior cingulate cortex. *Neuropsychopharmacology :*

official publication of the American College of Neuropsychopharmacology 2004, *29*, 171-178.

[20] Schmitt, A., Weber, S., Jatzko, A., Braus, D. F., Henn, F. A., Hippocampal volume and cell proliferation after acute and chronic clozapine or haloperidol treatment. *Journal of neural transmission* 2004, *111*, 91-100.

[21] Vernon, A. C., Natesan, S., Modo, M., Kapur, S., Effect of chronic antipsychotic treatment on brain structure: a serial magnetic resonance imaging study with ex vivo and postmortem confirmation. *Biological psychiatry* 2011, *69*, 936-944.

[22] Vernon, A. C., Crum, W. R., Lerch, J. P., Chege, W.*, et al.*, Reduced Cortical Volume and Elevated Astrocyte Density in Rats Chronically Treated with Antipsychotic Drugs-Linking Magnetic Resonance Imaging Findings to Cellular Pathology. *Biological psychiatry* 2013.

[23] Altar, C. A., in: Burger, A., Abraham, D. J. (Eds.), *Burger's medicinal chemistry and drug discovery*, Wiley and Sons, Hoboken 2003, pp. 599-672.

[24] Altar, C. A., Vawter, M. P., Ginsberg, S. D., Target identification for CNS diseases by transcriptional profiling. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2009, *34*, 18-54.

[25] Bachmann, R. F., Schloesser, R. J., Gould, T. D., Manji, H. K., Mood stabilizers target cellular plasticity and resilience cascades: implications for the development of novel therapeutics. *Molecular neurobiology* 2005, *32*, 173-202.

[26] Gould, T. D., Quiroz, J. A., Singh, J., Zarate, C. A., Manji, H. K., Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. *Molecular psychiatry* 2004, *9*, 734-755.

[27] Lewis, D. A., Antipsychotic medications and brain volume: do we have cause for concern? *Archives of general psychiatry* 2011, *68*, 126-127.

[28] Halim, N. D., Weickert, C. S., McClintock, B. W., Weinberger, D. R., Lipska, B. K., Effects of chronic haloperidol and clozapine treatment on neurogenesis in the adult rat hippocampus. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2004, *29*, 1063-1069.

[29] Hakansson, K., Pozzi, L., Usiello, A., Haycock, J.*, et al.*, Regulation of striatal tyrosine hydroxylase phosphorylation by acute and chronic haloperidol. *The European journal of neuroscience* 2004, *20*, 1108-1112.

[30] Lipska, B. K., Khaing, Z. Z., Weickert, C. S., Weinberger, D. R., BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs. *The European journal of neuroscience* 2001, *14*, 135-144.

[31] Farrelly, L. A., Dicker, P., Wynne, K., English, J.*, et al.*, Adolescent Risperidone treatment alters protein expression associated with protein trafficking and cellular metabolism in the adult rat prefrontal cortex. *Proteomics* 2014, *14*, 1574-1578.

[32] Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 1976, *72*, 248-254.

[33] Vale, R. D., The molecular motor toolbox for intracellular transport. *Cell* 2003, *112*, 467-480.

[34] Glantz, L. A., Lewis, D. A., Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch.Gen.Psychiatry* 2000, *57*, 65-73.

[35] Ellison-Wright, I., Bullmore, E., Anatomy of bipolar disorder and schizophrenia: a meta-analysis. *Schizophrenia research* 2010, *117*, 1-12.

[36] Connor, C. M., Crawford, B. C., Akbarian, S., White matter neuron alterations in schizophrenia and related disorders. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience* 2011, *29*, 325-334.

[37] Arnold, S. E., Lee, V. M., Gur, R. E., Trojanowski, J. Q., Abnormal expression of two microtubule-associated proteins (MAP2 and MAP5) in specific subfields of the hippocampal formation in schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America* 1991, *88*, 10850-10854.

[38] Rioux, L., Ruscheinsky, D., Arnold, S. E., Microtubule-associated protein MAP2 expression in olfactory bulb in schizophrenia. *Psychiatry research* 2004, *128*, 1-7.

[39] Somenarain, L., Jones, L. B., A comparative study of MAP2 immunostaining in areas 9 and 17 in schizophrenia and Huntington chorea. *Journal of psychiatric research* 2010, *44*, 694-699.

[40] Solis-Chagoyan, H., Calixto, E., Figueroa, A., Montano, L. M.*, et al.*, Microtubule organization and L-type voltage-activated calcium current in olfactory neuronal cells obtained from patients with schizophrenia and bipolar disorder. *Schizophrenia research* 2013, *143*, 384-389.

[41] Brown, J. W., Rueter, L. E., Zhang, M., Predictive validity of a MK-801-induced cognitive impairment model in mice: implications on the potential limitations and challenges of modeling cognitive impairment associated with schizophrenia preclinically. *Progress in neuro-psychopharmacology & biological psychiatry* 2014, *49*, 53-62.

[42] English, J. A., Dicker, P., Focking, M., Dunn, M. J., Cotter, D. R., 2-D DIGE analysis implicates cytoskeletal abnormalities in psychiatric disease. *Proteomics* 2009, *9*, 3368-3382.

[43] Benitez-King, G., Ramirez-Rodriguez, G., Ortiz, L., Meza, I., The neuronal cytoskeleton as a potential therapeutical target in neurodegenerative diseases and schizophrenia. *Current drug targets. CNS and neurological disorders* 2004, *3*, 515-533.

[44] Corena-McLeod, M., Walss-Bass, C., Oliveros, A., Gordillo Villegas, A.*, et al.*, New model of action for mood stabilizers: phosphoproteome from rat pre-frontal cortex synaptoneurosomal preparations. *PloS one* 2013, *8*, e52147.

[45] Duda, D. M., Scott, D. C., Calabrese, M. F., Zimmerman, E. S.*, et al.*, Structural regulation of cullin-RING ubiquitin ligase complexes. *Current opinion in structural biology* 2011, *21*, 257-264.

[46] Dubiel, D., Gierisch, M. E., Huang, X., Dubiel, W., Naumann, M., CAND1-dependent control of cullin 1-RING Ub ligases is essential for adipogenesis. *Biochimica et biophysica acta* 2013, *1833*, 1078-1084.

[47] Modica-Napolitano, J. S., Lagace, C. J., Brennan, W. A., Aprille, J. R., Differential effects of typical and atypical neuroleptics on mitochondrial function in vitro. *Archives of pharmacal research* 2003, *26*, 951-959.

[48] Prince, J. A., Yassin, M. S., Oreland, L., Neuroleptic-induced mitochondrial enzyme alterations in the rat brain. *The Journal of pharmacology and experimental therapeutics* 1997, *280*, 261-267.

[49] Tabb, D. L., What's driving false discovery rates? *Journal of proteome research* 2008, *7*, 45-46.

[50] Frost, D. O., Page, S. C., Carroll, C., Kolb, B., Early exposure to haloperidol or olanzapine induces long-term alterations of dendritic form. *Synapse (New York, N.Y.)* 2010, *64*, 191-199.

[51] van der Marel, K., Klomp, A., Meerhoff, G. F., Schipper, P.*, et al.,* Long-term oral methylphenidate treatment in adolescent and adult rats: differential effects on brain morphology and function. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2014, *39*, 263-273.

[52] Vizcaino, J. A., Cote, R. G., Csordas, A., Dianes, J. A.*, et al.*, The PRoteomics IDEntifications (PRIDE) database and associated tools: status in 2013. *Nucleic acids research* 2013, *41*, D1063-1069.

**Table 1: Top 5 IPA protein networks associated with chronic haloperidol treatment**

Network 1	**Associated network functions**: Cellular Assembly and Organization, Cellular Function and Maintenance, Tissue Development
	Number of 'focus molecules' contained in the network: 26
	IPA p-score: 49

	Network proteins: 26s Proteasome,60S ribosomal subunit;ACLY;Alpha tubulin Beta; Tubulin,CAND1,CAPZB,CCT3,DLST,DUSP3,ERK,FAM98B,GOT1,HSP,Hsp90, HSPA4,HSPA8,INPP1,NAP1L1,PP2A,PPP2CA,PSMA2,PSMB2,PSMD6,QDPR, RPL4,RPL7,RPL22,RPL31,RPLP1,SRR,STMN1,TUBB,Tubulin,TXNL
Network 2	**Associated network functions**: Cellular Function and Maintenance, Molecular Transport, Cell-To-Cell Signaling and Interaction
	Number of 'focus molecules' contained in the network: 24
	IPA p-score: 46
	Network proteins: AAK1,ADD3,ADRB,AGK,Ap2alpha,AP2B1,ATP1A2,ATP1B2,ATP1B3,BIN1,CA DPS,CASKIN1,Clathrin, Dynamin,ENDOD1,ERK1/2,Na+,K+ -ATPase,Na-k-atpase,NAPG,NCALD,NSFL1C,PTGDS,SEPT3,SEPT5,SEPT6,Septin,SH3GL3, SNAP25,Snare,STX1B,Syntaxin,TOLLIP,TPT1,TRIM2,Ubiquitin
Network 3	**Associated network functions**: Nucleic Acid Metabolism, Small Molecule Biochemistry, DNA Replication, Recombination, and Repair
	Number of 'focus molecules' contained in the network: 24
	IPA p-score: 45
	Network proteins: ATP5A1,ATP5B,ATP5O,ATP6V1F,ATPase,BCR (complex),CD3,CNBP,CRK,CTSB,ENO2,FAAH,FDPS,GLS,H+-transporting two-sector ATPase,H2AFX,HNRNPH3,HNRNPR,HSPA9,IL12 (complex),ILF2,Jnk,MAP2K1,MAP2K1/2,MDH2,PDIA6,PGK1,RNH1,RPS18,RP S19,Sapk,Secretase gamma,Sos,TCR,TUFM
Network 4	**Associated network functions**: Free Radical Scavenging, Small Molecule Biochemistry, Molecular Transport
	Number of 'focus molecules' contained in the network: 22
	IPA p-score: 40
	Network proteins: ACADL,Akt,ATP synthase,ATP2B2,C1QBP,Calcineurin protein(s),caspase,COX5A,Cycs,Cytochrome bc1,cytochrome C,cytochrome-c oxidase,DLAT,ETFA,F1 ATPase,HSPD1,Ikb,Mitochondrial complex 1,NADH Dehydrogenase,NADPH oxidase,NDUFA4,NDUFA11,NDUFB10,NDUFS1,NDUFS3,NOL3,peroxidase (miscellaneous),PRDX1,PRDX2,PRDX5,PRDX6,TST,UQCRB,UQCRC1,UQCR FS1
Network 5	**Associated network functions**: Cellular Assembly and Organization, Cellular Function and Maintenance, Cell Cycle
	Number of 'focus molecules' contained in the network: 23
	IPA p-score: 39
	Network proteins: ACOT7,ACTG1,Actin,ACTN1,ACTN4,ACTR1B,Alpha Actinin,Alpha catenin,CDC42,Cofilin,CTNNA2,DCTN2,DSTN,ECHS1,Erm,F Actin,GDA,GDI1,GDI2,GIT1,ICAM5,LASP1,Mlc,MYL6,MYO5B,NFkB (complex),Pak,PYGB,Ras homolog,Rho gdi,RHOC,Rock,SLC9A3R1,SORBS1,SYNPO

Legend: **Top 5 IPA protein networks associated with chronic haloperidol treatment.** IPA generates the Network using a proprietary algorithm, and adds proteins not contained in the original dataset. The p-score is calculated by IPA, and indicates the probability of finding a given number of focus molecules in a given network randomly selected from IPA's Global Molecular Network. The p-score is defined as: p-score = -$\log_{10}$ (p-value); the p-value is calculated by Fisher's exact test.
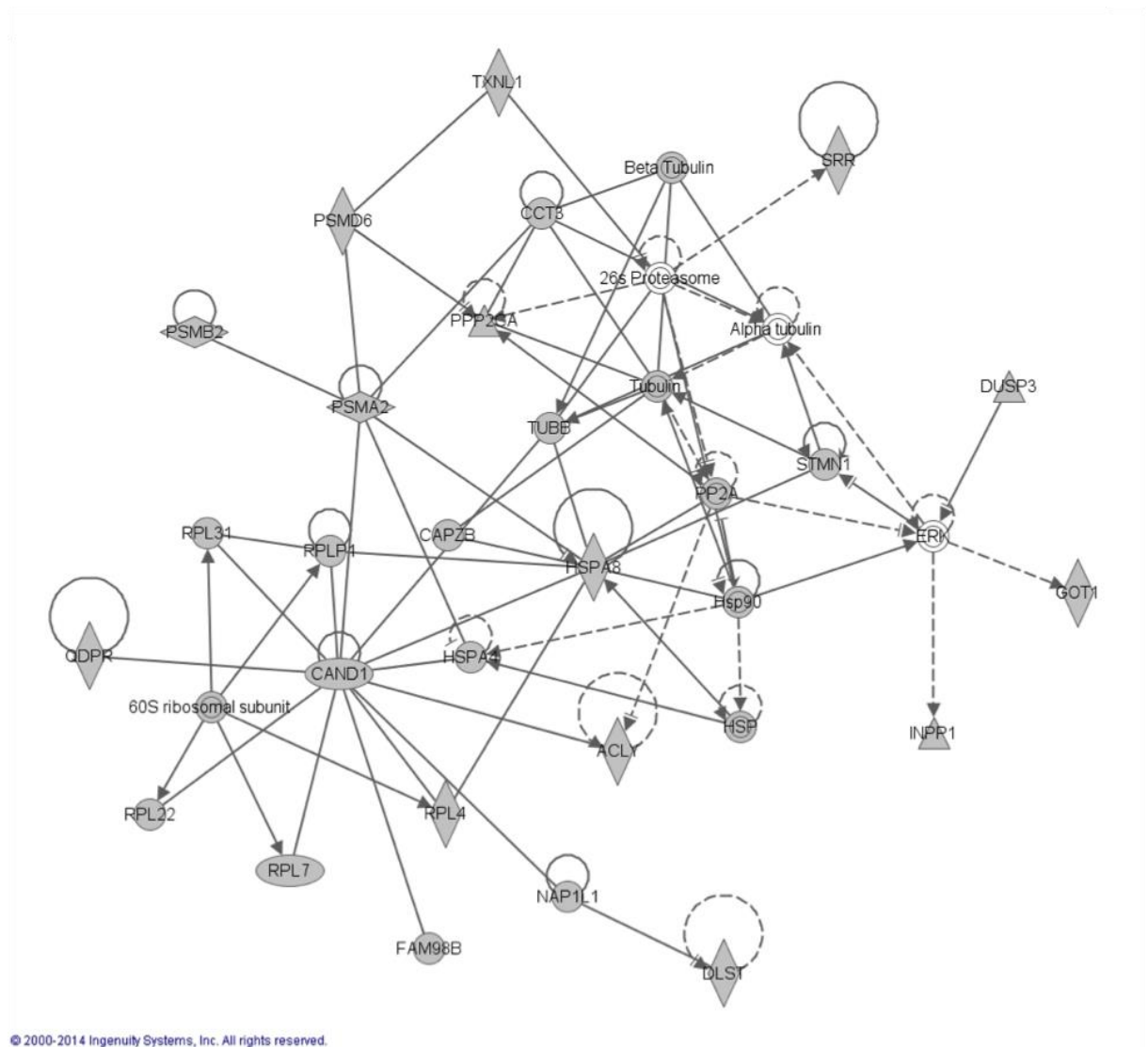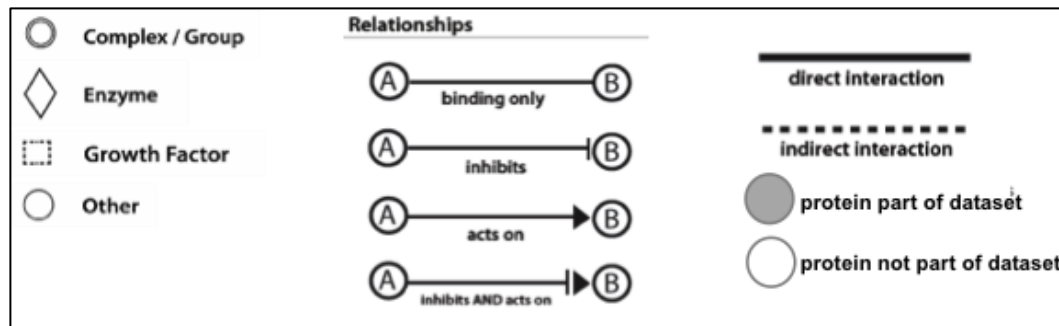
Legend Figure 1**: Graphic representation of mouse hippocampus protein network 1, identified by IPA as being affected by chronic haloperidol treatment.** Graphic representation of the top protein network in bipolar disorder Known direct and indirect interactions between network proteins, as well as the direction of the interaction, are indicated by thin arrows (activation) or thin blocked lines (inhibition). Central to the network are tubulin, heat shock cognate 71 kDa protein (HSPA8), and Cullin-associated Nedd8-dissociated protein 1 (CAND1).

Table 2: Top 5 IPA-identified canonical pathways affected by chronic haloperidol treatment

Name	p-value	IPA ratio
Oxidative Phosphorylation	3.13E-11	13/120 (0.108)
Mitochondrial Dysfunction	6.81E-09	13/215 (0.06)
Epithelial Adherens Junction Signaling	1.52E-07	11/154 (0.071)
Signaling by Rho Family GTPases	1.36E-05	11/263 (0.042)
Germ Cell – Sertoli Cell Junction Signaling	1.87E-05	9/169 (0.053)

Legend Table 2: The table lists the top 5 canonical pathways associated with schizophrenia, as identified by IPA. The Ratio divides the number of implicated proteins from the study by the total number of pathway proteins listed in the IPA knowledge base. The p-value indicates the likelihood of each Ratio occurring by chance.