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Molecular Validation of the Acute Phencyclidine Rat Model for Schizophrenia: Identification of Translational Changes in Energy Metabolism and Neurotransmission

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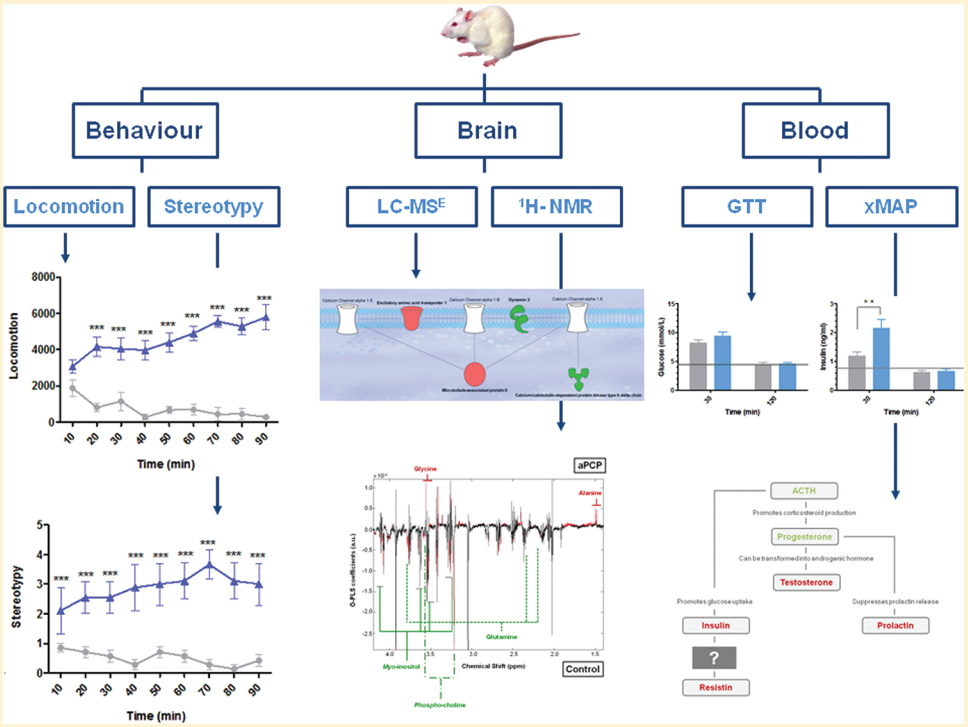
Supporting Information



ABSTRACT: Administration of the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) to rodents is widely used as preclinical model for schizophrenia. Most studies on this model employ methods investigating behavior and brain abnormalities. However, little is known about the corresponding peripheral effects. In this study, we analyzed changes in brain and serum molecular profiles, together with alterations in behavior after acute PCP treatment of rats. Furthermore, abnormalities in peripheral protein expression of first and recent onset antipsychotic free schizophrenia patients were assessed for comparison with the preclinical model. PCP treatment induced hyperlocomotion and stereotypic behavior, which have been related to positive symptoms of schizophrenia. Multiplex immunoassay profiling of serum revealed molecular abnormalities similar to those seen in first and recent onset, antipsychotic free schizophrenia patients. Also, increased insulin levels were detected after administration of a glucose tolerance test (GTT), consistent with previous studies showing changes in insulin signaling in patients with schizophrenia. Finally, schizophrenia-relevant alterations in brain molecules were found in the

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hippocampus and to a lesser extent in the frontal cortex using liquid-chromatography mass spectrometry and ^{1}H nuclear magnetic resonance spectroscopy. In conclusion, this study identified behavioral and molecular alterations in the acute PCP rat model, which are also observed in human schizophrenia. We propose that the corresponding changes in serum in both animals and patients may have utility as surrogate markers in this model to facilitate discovery and development of novel drugs for treatment of certain pathological features of schizophrenia.

KEYWORDS: *phencyclidine, rat, schizophrenia, multiplexed immunoassay, LC−MS, NMR, GTT,*

INTRODUCTION

Animal models are widely used in different areas of research to gain a better understanding of underlying disease mechanisms. The main objective is an attempt to mimic human conditions through face, construct, etiologic, and/or predictive validity.[1] One of the most prominent animal models for neuropsychiatric diseases is the phencyclidine (PCP) rat model. PCP is a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, which has been used to induce behavioral alterations in rodents, closely resembling specific symptoms and abnormalities, which have also been observed in schizophrenia patients.[2−5] Furthermore, it is known that PCP and other NMDA-receptor antagonists provoke schizophrenia-like symptoms in nonschizophrenic humans, and this can be reversed by antipsychotic medications.[6,7] Whereas chronic administration of PCP to rodents appears to induce persistent alterations mimicking the long-term impairments of schizophrenia such as cognitive defects and negative symptoms,[8] the acute treatment model bears a resemblance to the clinical picture of first-episode schizophrenia.[9] Preclinical research for schizophrenia and other neuropsychiatric disorders has focused mainly on analysis of behavioral readouts in relation to drug efficacy. However, translation of rodent phenotypical changes to behavioral abnormalities in humans is limited and can lead to erroneous findings. This results from the fact that animal models can only mirror certain behavioral traits of complex mental disorders. Furthermore, the lack of standardized behavioral protocols makes evaluation and comparison between different studies difficult. The most comprehensive studies have attempted to correlate behavioral changes with molecular effects in disease-relevant brain areas,[10−12] providing a link between behavior and underlying brain deficits. However, only a few studies investigating peripheral effects of PCP have been performed. Such studies are important, considering recent hypotheses that schizophrenia may be a systemic disorder.[13]

Previous investigations have attempted to gain a better understanding of peripheral abnormalities in schizophrenia by identifying molecular biomarkers in serum or plasma of patients.[14,15] It is hoped that such biomarkers can be used for diagnostic purposes and to provide novel insights into abnormalities of specific molecular pathways and cellular functions. Identification of translatable biomarkers in preclinical models would therefore enable the assessment of model validity for certain aspects of schizophrenia and support their use in drug discovery. Most importantly, translational blood serum changes can be used to track disease progression and response to pharmaceutical interventions.

Screening for peripheral biomarkers in first onset schizophrenia patients has shown that abnormalities in glucose metabolism are present in some patients even before the use of antipsychotic medications, and therefore, these are thought to be linked to disease pathology.[16−18] Identification of biomarkers associated with this phenomenon could lead to a means of stratifying patients prior to treatment and thus provide a means of facilitating more personalized therapies. However, in order to target such abnormalities in schizophrenia patients, animal models mimicking these defects must be established and validated. It is important that these models represent not only the behavioral aspects but also the molecular abnormalities found in the brain and circulation of patients.

In this study, we have characterized the acute PCP rat model of schizophrenia using a combination of behavioral and molecular analyses. The ultimate goal was to identify translatable molecular readouts to evaluate the validity of this model in relation to molecular changes seen in schizophrenia patients. Locomotor activity and stereotypic behaviors were measured to assess face validity of the model. Alterations in serum molecules were detected using multiplex immunoassay analysis, and changes in frontal cortex and hippocampal molecules were investigated using liquid chromatography−mass spectrometry (LC−MS) and ^{1}H nuclear magnetic resonance (NMR). In addition, PCP-treated rats were challenged using a glucose tolerance test in an attempt to recapitulate the metabolic abnormalities in schizophrenia and to determine any specific effects on glucose handling or insulin signaling.

MATERIALS AND METHODS

Animals and Housing Conditions

Male, adult Sprague−Dawley rats (Charles River, Margate, U.K.), weighing 280−300 g were housed in groups of four under standard laboratory conditions at 21 °C (±1 °C) on a 12 h/12 h light/dark cycle (lights on at 08:00). Experiments were conducted during the light cycle. Food and water were available ad libitum. All experiments were carried out in full compliance with the Home Office Guidance (U.K. Animals Scientific Procedures Act 1986) and the ethical policies of the Home Office.

Drugs and Solutions

Saline solution was 0.9% (w/v) sodium chloride in water (Fisher Scientific; Loughborough, U.K.). PCP hydrochloride was dissolved in 0.9% saline. Glucose (Sigma; Poole, U.K.) was dissolved in water at 1 mg/mL.

Treatment

Rats were treated with either PCP treatment or saline. PCP was administered once by subcutaneous injection at a dose of 5 mg/kg. Previous studies have shown that this dosage produces robust effects on locomotion[19] and causes molecular changes at the transcriptional level.[20] The control group received an equivalent volume of saline.

Human Samples

The human participants consisted of 250 first- and recent-onset schizophrenia patients and 230 controls matched for age, gender, and social status. Participants were recruited from the Departments of Psychiatry at the Universities of Cologne, Muenster, Magdeburg and Rotterdam. Schizophrenia was diagnosed based

on the Structured Clinical Interview for Diagnostic and Statistical Manual (DSM)-IV by psychiatrists following Good Clinical Practice guidelines. The subjects were all of the paranoid subtype (DSM-IV 295.30). Controls with a family history of mental disease or with other medical conditions such as type II diabetes and cardiovascular or autoimmune diseases were excluded. The medical faculty ethical committees of the respective research facilities approved the protocols of the study and clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was given in writing by all participants.

Behavioral Experiments

For assessment of locomotor activity, the IR Actimeter System from Panlab (Barcelona, Spain) was used. The system is composed of a 45 × 45 × 35 cm plastic box and 2 infrared beam frames. Each frame consists of 16 × 16 infrared beams for analyzing horizontal and vertical motion. Rats were handled for one week prior to the experimental day to facilitate adjustment to the environment and procedures. Two days prior to experiments, rats received habituation sessions of 30 min/day in the locomotion box. After a further 30 min of habituation on the experimental day, rats received subcutaneous injections of either PCP (n = 8) or saline (n = 8) and were placed back into the box. Locomotor activity was recorded for 90 min in 10 min intervals. Each beam break was indicated as one count. Stereotypic behavior was assessed simultaneously in 10 min intervals by an observer blinded to rat identity using a rating scale as described.[3]

Blood Analysis

Serum Preparation. Blood was collected from rats between 9:00 and 12:00 into S-Monovette 7.5 mL serum tubes (Sarstedt; Numbrecht, Germany) and left for 1.5 h at room temperature for clotting. The blood was centrifuged at 3000g for 15 min at 4 °C, and the resulting supernatants (sera) were stored in Low Binding Eppendorf tubes (Hamburg, Germany) at −80 °C.

Blood from schizophrenia and control subjects was collected between 8:00 and 12:00 into S-Monovette 7.5 mL serum tubes (Sarstedt). Samples were left at room temperature for 2 h for blood clotting. After centrifugation at 4000g for 5 min, serum supernatants were stored at −80 °C in Low Binding Eppendorf tubes.

Multiplex Immunoassay Profiling. Differences in protein expression were analyzed via multiplex immunoassay analysis at the Myriad-RBM Incorporated biomarker profiling facility (Austin, TX, U.S.A.). Serum analyses of PCP-treated (n = 8) and control (n = 8) rats were conducted using the Rodent MAP, Rat MetabolicMAP, and Rat KidneyMAP panels, comprising 89 molecular immunoassays. Sera from schizophrenia (n = 250) and control (n = 230) subjects were analyzed using similar multiplex immunoassays (Human DiscoveryMAP) comprising 181 assays. The methods have been described in detail previously.[21,22] Briefly, antibody−microsphere conjugates are incubated with the samples for binding of the targeted molecules. After washing, fluorescent reporter antibodies are added, which bind to different epitopes on the molecules. Unbound detection reagents are removed by washing prior to reading on a Luminex machine (Austin TX, U.S.A.). Within this instrument, the excitation beams of a red laser measures the unique fluorescent signature of each microsphere, and a green laser determines the amount of fluorescence generated in proportion to the concentration of the molecule in the sample. Data are acquired and reported in real-time, affording the ability to repeatedly measure the concentration of a given molecule in each sample. A combined list of 76 molecules, which could be measured in both the clinical and preclinical cohorts, was used to facilitate direct comparison of human and rodent data.

Glucose Tolerance Test

Rats were handled and received subcutaneous injections of saline for one week to reduce the influence of stress on blood glucose levels. After an overnight fast, PCP (5 mg/kg) or saline was administered subcutaneously on the experimental day. Following intraperitoneal injection of glucose solution (2 g/kg), blood glucose levels were determined at 30 and 120 min (n = 14) using the OneTouchUltraSmartMeter (LifeScan; High Wycombe, U.K.). Basal glucose concentrations were measured in rats that did not receive treatment (n = 24). After glucose determinations, rats were killed by decapitation and blood samples collected. Serum insulin concentrations were determined using an Ultra Sensitive Rat Insulin ELISA kit (Chrystal Chem Inc.; Downers Grove, IL, U.S.A.).

LC−MS Analysis of Brain Tissue

Thirty minutes after PCP (n = 10) or saline (n = 10) administration, rats were decapitated and brains dissected on ice. Frontal cortex and hippocampus tissue were stored at −80 °C. Tissue samples were added to fractionation buffer containing 7 M urea, 2 M thiourea, 4% chaps, 2% ASB14, 70 mM DTT, and protease inhibitor at a 5:1 (v/w) ratio.[23] After sonication and vortexing for 30 min, samples were centrifuged 3 min at 17 000g at 4 °C. Protein concentrations of the lysates were determined using a Bradford assay (Bio-Rad; Hemel Hempstead, U.K.). Protein (approximately 100 μg) was precipitated using acetone. After dissolving the precipitate in 50 mM ammonium bicarbonate, reduction of sulfhydryl groups was performed with 5 mM DTT at 60 °C for 30 min and alkylation was carried out using 10 mM iodacetamide and incubating in the dark at 37 °C for 30 min. Digestion of the proteins was conducted using trypsin at a 1:50 (w/w) ratio for 17 h at 37 °C, and reactions were stopped by the addition of 8.8 M HCl in a 1:60 (w/w) ratio.

Quality control (QC) samples were also prepared to monitor machine performance. For this, spare tissue samples were sonicated in fractionation buffer and centrifuged, and the resulting lysates were pooled and divided into several samples. Each QC sample underwent all experimental steps in parallel with the test samples.

Analysis of brain tissue samples was performed by LC−MS as described previously.[24] Adaptations to the protocols were made as described below. Samples and QCs were diluted in 0.1% formic acid to a final concentration of 0.12 μg/μL protein and analyzed in duplicate. Reverse phase chromatographic peptide separation was performed using a nanoACQUITY ultra performance liquid chromatography system (Waters Corporation; Milford, MA, U.S.A.). The system was comprised of a C18 trapping column (180 μm × 20 mm; 5 μm particle size) and a C18 BEH nanocolumn (75 μm × 200 mm; 1.7 mm particle size). The buffers were as follows: (A) H_2O + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid. Initial buffer concentrations were 3% B (97% A) followed by 3−30% B (90 min), 30−90% B (25 min), 90−97% B (5 min), constant 97% B (10 min), and 97−3% B (1 min). The LC analytical column was coupled online to a nanoESI emitter (7 cm length, 10 mm tip) on a quadrupole time-of-flight mass spectrometer (Q-TOF) PREMIER mass spectrometer (Waters corporation; Milford, MA, U.S.A.), and data were acquired in MS mode. Approximately 500 fmol/μL

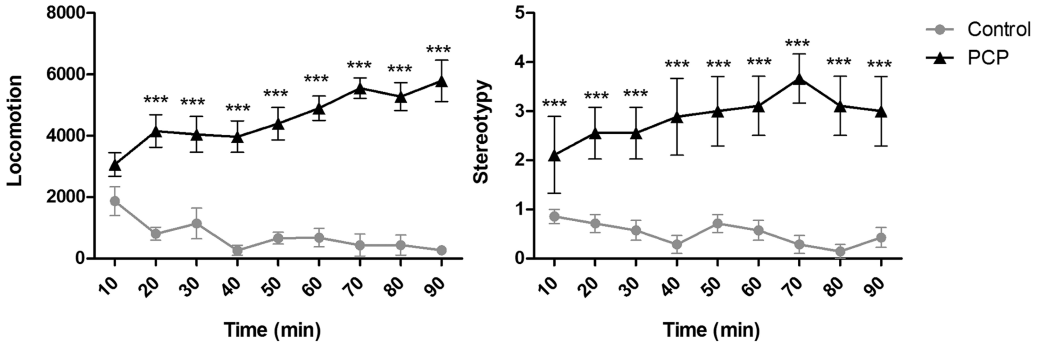

Figure 1. Locomotion and stereotypy assessment after acute PCP injection. (A) Locomotion was measured via movement through an infrared beam grid in locomotion chamber. The counts on the *Y* axis indicate the number of beam breaks. (B) PCP-induced stereotypic behavior relative to controls using a rating system of 0−5 as described in the materials and methods section. Data are expressed as mean ± SEM; *** $p < 0.001$.

Glu Fibrinopeptide B was infused every 30 s using a lock spray for external lock mass correction. The mass spectrometer was operated in V mode, and analyses were performed using positive nanoESI ion mode. Collision energy was 5 eV for low-energy scans and ramped from 17 to 40 eV for high-energy scans (cycle time 1.3 s). The low collision energy (MS) generates information about intact precursor ions, and the high collision energy (MSE) provides information about the peptide fragments.

^{1}H NMR Spectroscopic Analysis of Brain Tissue

Frontal cortex and hippocampus samples were extracted for aqueous molecules using acetonitrile/deionized water (2:3) solution in 4 mL capacity glass vials containing metal beads (Qiagen; Crawley, U.K.). Samples were homogenized using a tissue grinder (Qiagen) set at 30 Hz for 5 min. After removal of the beads, chloroform/methanol (3:1) was added and the vials vortexed until the two phases were homogeneous. The samples were centrifuged for 10 min at 1200*g* at 5 °C. The aqueous phase containing hydrophilic molecules was collected and left to dry overnight at room temperature to remove acetonitrile and methanol, and then freeze-dried at −70 °C. The samples were reconstituted in deuterium oxide and transferred into 5 mm NMR tubes for analysis. For each sample, a ^{1}H NMR spectrum was acquired at 600.13 MHz on a Bruker DRX-600 spectrometer (Bruker GmBH; Rheinstetten, Germany) at ambient temperature using the first increment of the Nuclear Overhauser Enhancement Spectroscopy pulse sequence (RD, $\pi/2-t1-\pi/2-tm-\pi/2$; TR = 3 s) for suppression of water resonance as described previously.[25] Complete spectra over the 0.20−10 ppm chemical shift range were processed. All spectra were phase- and baseline-corrected and calibrated using the reference resonance at 1.33 ppm (doublet corresponding to lactate CH_3 group) via the Topspin v2.0 software (Bruker; Billerica, MA, U.S.A.). Spectral variation due to water presaturation was removed by zeroing intensity values between 4.5 and 5.2 ppm. To account for concentration differences between samples, data were normalized to the total integrated peak area intensity by converting integral values to a percentage of summed integrals. The experiment was performed under blinded and randomized conditions.

Data Analysis

Behavioral data and glucose and insulin concentrations were analyzed using two-way analysis of variance (ANOVA) and post hoc Bonferroni tests. Analysis of a multiplex immunoassay of rat sera data was conducted using nonparametric Wilcoxon rank−sum tests. Before applying univariate statistics, normalization of data across the five clinical centers was carried out based on levels of each analyte in control samples. p values less than 0.05 were considered as statistically significant.

LC−MS data were processed using ProteinLynx Global Server (PLGS) v.2.4 (Waters Corporation; Milford, MA, U.S.A.) and Rosetta Elucidator v.3.3 (Rosetta Biosoftware; Seattle, WA, U.S.A.) for time and mass/charge alignment of mass spectrometer data as described previously.[26] The *Rattus norvegicus* complete proteome fasta sequence Integr8 database was used for the appropriate protein searches. Only proteins with a ≥2 peptide count were considered for further analyses.

Chemometric modeling of ^{1}H NMR data was performed as described.[27] Full resolution spectra were analyzed using projection to latent structure discriminant analysis (PLS-DA) and orthogonal (O)-PLS-DA [SIMCA P v12 (Umetrics AB; Umea, Sweden)] to identify molecules differing between PCP-treated and control rats and for removal of confounding factors. Data were modeled using MATLAB v6.5 (The Mathworks Inc.; Natick, MA, U.S.A.) and scripts proprietary to Imperial College, London, U.K.

RESULTS

Behavioral Assessment

Acute treatment of rats with PCP resulted in a persistent increase in locomotor activity over the 90 min test period, whereas locomotion of control rats decreased (Figure 1). Two-way ANOVA revealed significant changes in the treatment-time interaction ($F = 6.235$, $p < 0.0001$) and main effect of treatment ($F = 94.22$, $p < 0.0001$). Post hoc tests indicated significant hyperlocomotion of PCP rats from 20 to 90 min after PCP injection ($p < 0.0001$). Stereotypic behavior was assessed using a rating system from 0 (inactivity) to 5 (dyskinetic extension and flexion of limbs, head, and neck; gagging; weaving). PCP-treated rats had ratings ranging from 2.0 to 3.7 over the 90 min assessment period, whereas control rats had scores from 0 to 1.0. As with locomotion activity, the treatment-time interaction ($F = 5.735$, $p < 0.0001$) and main effect of treatment ($F = 235.0$, $p < 0.0001$) were significantly different. Post hoc tests showed significant increases in stereotypic behavior in PCP-treated rats at all time points ($p < 0.0001$).

Brain Analysis

Liquid Chromatography−Mass Spectrometry. LC−MS analysis of one hemisphere of frontal cortex from control and PCP treated rats resulted in identification of 373 unique proteins, using criteria defined in the materials and methods section. Only one of these, the enzyme sarcoplasmic/endoplasmic

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Table 1. Molecular Changes in the Hippocampus after 5 mg/kg Administration of PCP[a]

uniprot KB number	gene name	protein name	function	*p* value	FC	RSD QCs (%)
Cytoplasm						
▲B2GV06[#]	SCOT1_RAT	3-oxoacid-CoA transferase 1	enzyme	0.0473	1.49	26
▲O35303[#]	DNM1L_RAT	dynamin-1-like protein	enzyme	0.0431	1.35	9
▲P50137	TKT_RAT	transketolase	enzyme	0.0173	1.29	15
▲P85515	ACTZ_RAT	alpha-centractin	other	0.0457	1.20	17
▲P15791	KCC2D_RAT	calcium/calmodulin-dependent protein kinase type II delta chain	kinase	0.0369	1.18	12
▲P08461*	ODP2_RAT	dihydrolipoamideacetyltransferase component of PDC	enzyme	0.0452	1.15	9
▲O35095*	NCDN_RAT	neurochondrin	other	0.0286	1.13	6
▼P80254*	DOPD_RAT	D-dopachrome decarboxylase	enzyme	0.0319	−1.11	11
▼P11348	DHPR_RAT	dihydropteridine reductase	enzyme	0.0245	−1.13	10
▼Q9EQX9*	UBE2N_RAT	ubiquitin-conjugating enzyme E2 N; EC = 6.3.2.19	enzyme	0.0452	−1.13	11
▼Q62658	FKB1A_RAT	peptidyl-prolyl cis−trans isomerase FKBP1A	enzyme	0.0376	−1.13	12
▼Q63560*	MAP6_RAT	microtubule-associated protein 6	other	0.0414	−1.14	8
▼P85970	ARPC2_RAT	actin-related protein 2/3 complex subunit 2	other	0.0140	−1.17	20
▼Q7M0E3	DEST_RAT	destrin	other	0.0257	−1.24	43
Plasma Membrane						
▲P39052*	DYN2_RAT	dynamin-2	enzyme	0.0312	1.13	3
▲P54708	AT12A_RAT	potassium-transporting ATPase alpha chain 2	transporter	0.0372	1.12	7
▼Q9Z270*	VAPA_RAT	VAMP associated protein A	other	0.0157	−1.23	9
▼P24942*	EAA1_RAT	excitatory amino acid transporter 1	transporter	0.0123	−1.25	12
Nucleus						
▼O88767*	PARK7_RAT	protein DJ-1	enzyme	0.0080	−1.11	5

[a] *P* values of <0.05 were considered as statistically significant. Proteins indicated with * or [#] were found to be changing with a *p* value of <0.05 or <0.1, respectively, in a separate study involving chronic treatment of rats with PCP (Wesseling et al., manuscript in preparation). The biological functions of these molecules were listed according to annotations in the UniProt database (www.uniprot.org), and the relative standard deviation (RSD) for the measurement of each protein is given. The RSD was determined using quality controls that have been prepared and analyzed alongside the PCP and control samples.

reticulum calcium ATPase 2, was found to be significantly altered (*p* value,0.0379; FC, 1.12; peptide count > 1).

LC−MS analysis of extracts of one hippocampus from control and PCP treated rats resulted in identification of 449 unique proteins. Thirty-two of these proteins were found to be altered significantly between the two groups, with 19 proteins having fold changes >10% (Table 1).The results were also compared to those obtained from analysis of chronically treated PCP (5 mg/kg) rat hippocampus (Wesseling et al.; manuscript in preparation) to determine any similarities or differences between the two protocols (Table 1).

In silico pathway analysis of the uploaded molecules using the Ingenuity Pathways Knowledge Base showed that the top function associated with the most significant interaction network was molecular transport (Figure S1, Supporting Information). In addition, this analysis showed that the top disease category associated with the altered proteins was neurological disorders, and the top canonical pathway was clathrin-mediated endocytosis signaling.

^{1}H NMR Spectroscopy. Analogous to the LC−MS studies, analysis was performed on the frontal cortex and hippocampus using O-PLS-DA on full-resolution data. Figure 2 shows the resulting O-PLS-DA plot of the hippocampus depicting the molecules influential to the separation between control and PCP treated rats. The goodness of fit and prediction for the spectral data revealed brain region-specific molecular changes. In both frontal cortex and hippocampus, 5 molecules were differentially expressed after PCP treatment, with one metabolite, phosphocholine, decreased in both brain areas (Table 2).

Serum Analysis

Multiplex Immunoassay. To identify differences in the levels of serum molecules after acute PCP treatment, samples were measured by multiplex immunoassays as described in the materials and methods section. Data quality was assessed using principal component analysis (PCA), and no batch effects or outliers were detected (Figure S2, Supporting Information). The analysis revealed that 19 of the 92 molecules tested were present at significantly different levels between PCP and control rats (Table 3). Of these, 16 molecules were decreased after acute PCP treatment. Prolactin (ratio = 0.12), glutathione *S*-transferase alpha (GST alpha) (ratio = 0.22), and testosterone (ratio = 0.54) showed decreases of more than 1.8-fold. Only three molecules were found with increased levels in response to PCP treatment, and these were serum glutamic-oxaloacetic transminase (SGOT) (ratio = 1.60), progesterone (ratio = 1.38), and adrenocorticotrophic hormone (ACTH) (ratio = 1.32).

The list of molecules showing significant alterations after acute PCP administration in rats was compared to the same analysis of serum from schizophrenia and control subjects as described in the materials and methods section. In order to map and cross-compare human with rodent data, only those 76 molecules that could be measured in both the clinical and preclinical cohorts were tested (Table S1, Supporting Information). Of these, 28 molecules were altered between schizophrenia patients and controls, and 7 of these (all proteins) were changed in response to PCP treatment in the rat model (Figure S3, Supporting Information). The overlapping proteins between rodent and human data were tissue inhibitor of metalloproteinase 1 (TIMP-1), cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), insulin, glutathione *S*-transferase alpha (GST-alpha), prolactin, and serum gutamic-oxaloacetic transaminase (SGOT) (Table 4). The levels of all altered proteins apart from SGOT were decreased after acute PCP treatment, whereas all of these proteins were increased in schizophrenia patients.

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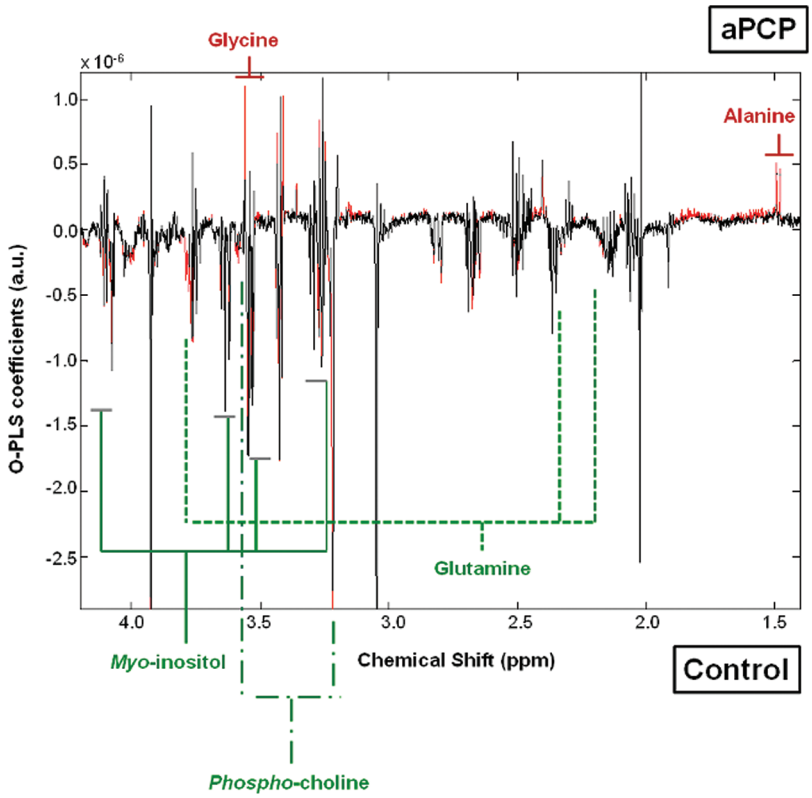

Figure 2. Multivariate statistical analysis of NMR spectra of soluble rat hippocampus extracts after PCP treatment. The loadings coefficient plot shows spectral descriptors presenting differences between the control and treatment groups. Molecules found to be altered following PCP treatment are represented as red spectra and highlighted in red (increased) and green (decreased). The metabolite ID's were confirmed by 2D NMR and statistical total correlation spectroscopy.[73]

Table 2. Summary of PCP-Induced Molecular Changes in Different Rat Brain Regions[a]

metabolite	chemical shift (ppm)	frontal cortex r^2	hippocampus r^2
alanine	1.48 (d)		▲ (0.32)
glycine	3.56 (s)		▲ (0.28)
inosine	3.85 (dd)	▲ (0.25)	
unknown	3.17 (t/m)	▲ (0.21)	
phosphocreatine	3.93 (s)	▲ (0.37)	
scylloinositol	3.26 (s)	▲ (0.24)	
glutamine	3.78 (m)		▼ (0.34)
	2.15 (m)		▼ (0.32)
	2.46 (m)		▼ (0.36)
myoinositol	4.06 (t)		▼ (0.34)
	3.63 (t)		▼ (0.35)
	3.53 (dd)		▼ (0.29)
	3.28 (t)		▼ (0.31)
phosphocholine	3.58 (t)	▼ (0.28)	▼ (0.41)
	3.22 (s)	▼ (0.27)	▼ (0.30)

[a]The table shows the regions affected by acute 5 mg/kg PCP treatment with the responding correlation coefficients (r^2). ▲ = increased; ▼ = decreased.

Glucose Tolerance Test. The hormonal alterations identified by this analysis indicated abnormalities in insulin secretion in PCP-treated rats. This was examined further by incorporation of a glucose tolerance test into the experimental protocol and testing for differences in the insulin response. After administration of PCP or saline, glucose (2 g/kg) was injected and serum levels of glucose and insulin were determined at 0, 30, and 120 min. This showed that glucose concentrations did not differ between the PCP and saline groups at any of the time points measured (treatment-time interaction, $F = 0.7822$, $p = 0.4611$; treatment, $F = 1.528$, $p = 0.2203$) (Figure 3A). However, insulin levels were elevated significantly at the 30 min time point in the PCP group relative to the saline controls (Figure 3B) (treatment-time interaction, $F = 5.656$, $p = 0.0052$; treatment, $F = 7.187$, $p = 0.0091$), suggesting that acute PCP treatment may induce a transient change in insulin signaling. The finding of increased insulin levels 30 min after a high glucose challenge was reproduced ($p = 0.01$) using a different batch of animals ($n = 7$ per group) to validate the results (Figure S4, Supporting Information).

■ DISCUSSION

There is currently only limited understanding of the underlying disease mechanisms of schizophrenia. The use of animal models can only address certain aspects of this complex human disorder. Correlations to human schizophrenia patients have been made mainly in terms of behavioral abnormalities or similarities in brain functional abnormalities, which cannot be easily translated between clinical and preclinical studies. However, valid animal models for neuropsychiatric disorders cannot be achieved without translational correlates and readouts.[28] Thus, increasing our knowledge about the etiology and pathophysiological mechanisms of schizophrenia will guide the development of better models. Furthermore, it is unlikely that a single animal model will reflect all aspects associated with complex psychiatric disorders such as schizophrenia. Therefore, a key aim in schizophrenia research should be to correlate behavioral, structural, and

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Table 3. Analysis of Protein Levels in Serum Using Multiplexed Immunoassay of PCP Treated (n = 8) and Control (n = 8) Rats[a]

molecule	p value	ratio change	function
▲ serum glutamic-oxaloacetic transminase	0.028	1.60	enzyme
▲ progesterone	0.001	1.38	hormone
▲ Adrenocorticotrophic hormone	0.006	1.32	hormone
▼ stem cell factor	0.014	0.92	signal transduction
▼ tissue inhibitor of metalloproteinase 1	0.010	0.89	cell growth
▼ beta-2 microglobulin	0.015	0.86	immune response
▼ cystatin C	0.015	0.86	protein metabolism
▼ resistin	0.021	0.86	hormone
▼ insulin like growth factor 1	0.005	0.86	growth factor
▼ neutrophil gelatinase-associated lipocalin	0.015	0.85	immune response
▼ macrophage colony stimulating factor	0.021	0.85	signal transduction
▼ vascular endothelial growth factor	0.002	0.83	growth factor
▼ interferon gamma-induced protein 10	0.044	0.83	immune response
▼ insulin	0.018	0.76	hormone
▼ macrophage inflammatory protein 1 beta	0.006	0.75	signal transduction
▼ tumor necrosis factor alpha	0.005	0.72	signal transduction
▼ testosterone	0.050	0.54	hormone
▼ glutathione S-transferase alpha	0.002	0.22	energy metabolism
▼ prolactin	<0.001	0.12	hormone

[a]The ratio change was calculated as PCP/control. The biological functions of these molecules were listed according to annotations in the UniProt database (www.uniprot.org).

Table 4. Overlap of Significant Serum Molecules[a]

	acute PCP			schizophrenia patients		
molecule	p value		ratio change	p value		ratio change
serum glutamic-oxaloacetic transaminase	0.028	▲	1.60	0.048	▲	1.09
tissue inhibitor of metalloproteinase 1	0.010	▼	0.89	<0.001	▲	1.09
cystatin c	0.015	▼	0.86	0.001	▲	1.09
neutrophil gelatinase-associated lipocalin	0.015	▼	0.85	0.005	▲	1.22
insulin	0.018	▼	0.76	0.031	▲	1.58
glutathione S-transferase alpha	0.002	▼	0.22	0.004	▲	1.52
prolactin	<0.001	▼	0.12	0.004	▲	1.80

[a]Serum proteins that were significantly changed after acute PCP treatment in rats and in first-onset schizophrenia patients [adapted from Schwarz et al.[72]].

molecular biomarkers to facilitate translational development of valid animal models that track specific deficits of this disorder. In this way, molecular changes could potentially be used in conjunction with relevant models as surrogate markers for response in drug discovery applications.

This is the first global molecular profiling study of the acute PCP rat model, which has attempted to integrate behavioral changes with molecular alterations in serum and brain tissues. In particular, this study has resulted in identification of molecular changes showing overlap of this model to metabolic imbalances in first or recent onset, antipsychotic-free schizophrenia patients.

Analysis of hippocampal extracts using LC−MS led to identification of altered proteins associated with synaptic molecular transport. Approximately 60% of these proteins were found to be altered after LC−MS analysis of hippocampi from rats treated chronically with PCP, suggesting that both models involved effects on similar pathways. In the acute model, we found that the most significant effects appeared to involve proteins associated with clathrin mediated endocytosis (CME) signaling, consistent with recent findings from studies on schizophrenia and bipolar disorder.[29,30] Clathrin mediates intracellular vesicle transport is involved in synaptic vesicle recycling.[31] Another synaptic protein altered by PCP treatment was the excitatory amino acid transporter-1, which is involved in the malate−aspartate shuttle of glial cells. Previous studies have shown that knockout (KO) of EAAT1 in mice induced locomotor hyperactivity, which was exaggerated after administration of MK-801, another noncompetitive NMDA receptor antagonist, and ameliorated by administration of the antipsychotic haloperidol or the mGlu2/3 agonist LY-379 268.[32] The clinical importance of EAAT1 in schizophrenia has been postulated in numerous studies, which have shown dysregulation of glutamate transporters in postmortem brain analyses,[33−35] supporting use of the PCP rat as a biologically relevant model for schizophrenia. Further evidence for the applicability of this model comes from reports showing that treatment with the typical and atypical antipsychotics haloperidol and olanzapine resulted in proteomic changes in the frontal cortex, which normalize PCP-induced alterations (increased actin-related protein concentrations after olanzapine treatment and decreased dynamin 2 levels after haloperidol treatment).[36] Furthermore, it has been shown that chronic clozapine treatment in human schizophrenia patients increases VAMP-1 concentrations, which could suggest a normalization effect on the observed decreased levels of the VAMP associated protein A.[37]

Only one significantly altered protein was identified in the frontal cortex. The reason for the low detection rate might be due to technical and sensitivity problems when acquiring the data. Alternatively, this could suggest that the effects of PCP on the cortex are more subtle than those in the hippocampus.

The results of the ^{1}H NMR spectroscopy analyses showing decreased glutamine levels supported our findings of reduced levels of EAAT1 in the hippocampus. This is interesting as previous studies have shown that first-onset antipsychotic nave schizophrenia patients have a dysfunctional glutamate−glutamine cycle.[38] In addition, we identified changes in other molecules we have already associated with the mechanism of action of therapeutic interventions in schizophrenia, including glycine[39] and alanine.[40] Studies on myoinositol levels have not been conclusive. However, our results showing altered phosphocholine levels in both brain regions analyzed are consistent with those of a ^{1}H MRS study of schizophrenia patients.[41]

In the frontal cortex, we found decreased phosphocholine concentrations with increased levels of phosphocreatine, scylloinositol, inosine, and a nonidentified molecule. In a recent ^{1}H MRSI study, researchers found that severity of formal thought disorder correlated with creatine and phosphocreatine levels in schizophrenia.[42] In contrast to our findings, one study found no differences in scylloinositol levels in postmortem brains of schizophrenia patients nor in unipolar and bipolar depression patients.[43]

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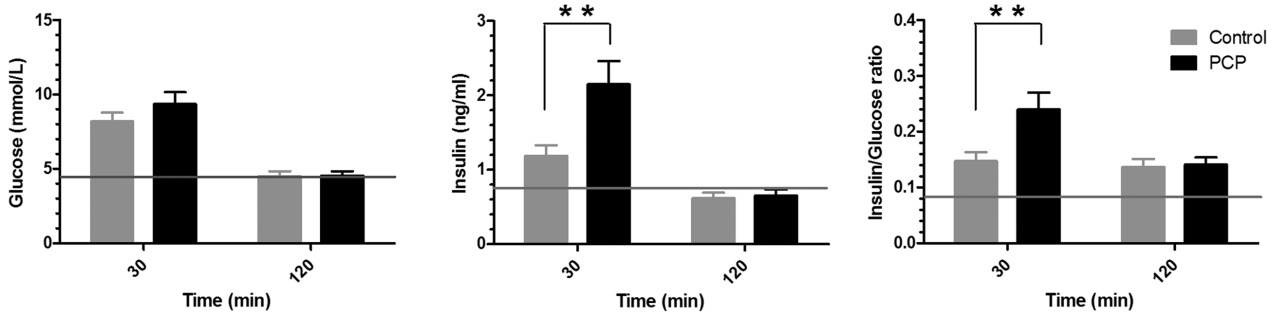

Figure 3. Effect of PCP on glucose tolerance and insulin levels in PCP treated (n = 14) and control (n = 14) rats. After administration of PCP, glucose (2 g/kg) was injected (ip) and (A) glucose and (B) insulin were determined at 30 and 120 min. (C) The insulin/glucose ratio was plotted at each time point.

Multiplex immunoassay profiling of serum resulted in the identification of 7 molecules (TIMP-1, cystatin C, NGAL, insulin, GST-alpha, prolactin, and SGOT), which were altered in both rodent and human samples. TIMP-1 is suggested to be involved in neuronal plasticity in learning and memory processes,[44,45] and GST-mu has already been linked to schizophrenia and major depressive disorder.[46] Furthermore, GST-mu is involved in cellular protection against oxidative stress,[47] which is known to be involved in schizophrenia.[48] Abnormal functioning of the glucose-regulating hormone insulin has been implicated in schizophrenia pathophysiology for decades.[49] Impaired insulin function after antipsychotic medication and the high incidence of metabolic disorders in schizophrenic patients have made this a molecule of interest for further investigations.[50,51] We also found changes in prolactin, consistent with previous findings and suggesting involvement of PCP in dopamine D2 receptor blockage.[52,53] Altered levels in cystatin C and NGAL levels have been observed in both Alzheimer's disease and mild cognitive impairment,[54,55] supporting their importance as possible disease markers in neuropsychiatric disorders. Apart from SGOT, all of the other proteins showed opposite directional changes between human and rodent samples. A possible explanation for this could be that the molecular changes found in the PCP model represent acute effects, rather than long-term, adaptive alterations as found in schizophrenia patients.

Interestingly, acute PCP treatment appeared to have a marked influence on endocrine function as six of the 19 significantly altered proteins in the rat model were hormones (Figure S5, Supporting Information). Apart from insulin and prolactin, which were discussed above, ACTH, testosterone, progesterone, and resistin were also affected. Increased levels of ACTH after PCP administration have already been reported.[56] ACTH is secreted in response to biological stress and promotes increased production and secretion of corticosteroids and related molecules, such as progesterone. Progesterone in turn has been shown to suppress prolactin release, consistent with our findings of reduced prolactin concentrations after PCP administration.[57] Additionally, ACTH is thought to have intrinsic hypoglycaemic activity as it can promote hepatic glucose uptake, regardless of the presence of insulin.[58] Another hormone altered after PCP administration was resistin, which is linked to insulin resistance as seen in obesity and type II diabetes.[59] The exact mechanism of action of resistin is not known, but several studies have demonstrated an association of this protein to these medical conditions.[60,61]

The increase found in insulin levels at the 30 min time point of the glucose tolerance test suggested that these rats had a transient impairment in metabolic stress adaptation. Glucose tolerance tests are used typically as a measure of peripheral energy metabolism status in the assessment of various metabolic disorders such as type II diabetes mellitus and insulin resistance,[62] and schizophrenia patients are at high risk for developing such conditions.[63] In addition, antipsychotic treatment is known to induce these abnormalities.[64] However, we recently identified altered levels of insulin-related molecules in first and recent onset schizophrenia patients, proposing a dysregulation in insulin signaling even before medication has been initiated,[16] and other studies have demonstrated insulin resistance in similar antipsychotic free patients.[18,65] The potential link between neuropsychiatric diseases and metabolic abnormalities is consistent with reports that the db/db mouse, a model for diabetes, obesity, and dyslipidemia, shows signs of depression, psychosis–like symptoms, and anxiolytic behavior.[66] Also, follow-up studies on this model have revealed normalization of the depression symptoms after treatment with the antidiabetic drug rosiglitazone.[67]

The changes in insulin signaling and glucose metabolism in mediating the effects on hormones and metabolites in the PCP model and in schizophrenia are most likely due to the fact that these pathways regulate the activity of most cells of the body including those of the diffuse neuroendocrine system. For example, Guest et al. showed recently that changes in insulin levels are also associated with changes in levels of other hormones such as progesterone, adrenocorticotrophic hormone, and prolactin, as we found here in the case of the PCP-treated rats.[68] Also, Cardoso et al. showed that acute streptozotocin-induced hyperglycemia and insulin-induced hypoglycaemia resulted in altered levels of cortical glutamine levels as we have found here.[69] A major advantage of using serum rather than brain tissue for translational studies in humans is that serum can be obtained easily by minimally invasive procedures. This also circumvents problems associated with use of postmortem brain tissues, such as confounding effects of long-term medication over the lifetime of the subjects.[70,71] Most importantly, if serum changes in schizophrenia patients are proven to track disease progression or treatment responses, which can be recapitulated in rodent models, the development of novel drug discovery approaches could be facilitated. In this way, molecular changes could be used in conjunction with the most robust models as surrogate markers of response.

■ ASSOCIATED CONTENT

Supporting Information

Combined human and rodent xMAP list; ingenuity pathway analysis of rat hippocampal proteomic changes after treatment

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with 5 mg/kg PCP; data quality assessed by PCA; overlap of serum molecular changes between human and rat studies; increased insulin concentrations after glucose challenge in the 5 mg/kg PCP rat model; hormonal imbalance after acute PCP administration. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Young, J. W.; Zhou, X.; Geyer, M. A. Animal models of schizophrenia. *Curr. Top. Behav. Neurosci.* **2010**, *4*, 391−433.

(2) Nabeshima, T.; Yamaguchi, K.; Yamada, K.; Hiramatsu, M.; Furukawa, H.; Kameyama, T. Phencyclidine-induced stereotyped behaviors in rats following specific neurotoxin lesions of the striatum. *Eur. J. Pharmacol.* **1983**, *93* (3−4), 229−234.

(3) Sturgeon, R. D.; Fessler, R. G.; Meltzer, H. Y. Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. *Eur. J. Pharmacol.* **1979**, *59* (3−4), 169−179.

(4) Sams-Dodd, F. Distinct effects of D-amphetamine and phencyclidine on the social behaviour of rats. *Behav. Pharmacol.* **1995**, *6* (1), 55−65.

(5) Egerton, A.; Reid, L.; McGregor, S.; Cochran, S. M.; Morris, B. J.; Pratt, J. A. Subchronic and chronic PCP treatment produces temporally distinct deficits in attentional set shifting and prepulse inhibition in rats. *Psychopharmacology* **2008**, *198* (1), 37−49.

(6) Allen, R. M.; Young, S. J. Phencyclidine-induced psychosis. *Am. J. Psychiatry* **1978**, *135* (9), 1081−1084.

(7) Javitt, D. C.; Zukin, S. R. Recent advances in the phencyclidine model of schizophrenia. *Am. J. Psychiatry* **1991**, *148* (10), 1301−8.

(8) Jentsch, J. D.; Roth, R. H. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **1999**, *20* (3), 201−25.

(9) Adell, A.; Jimenez-Sanchez, L.; Lopez-Gil, X.; Romon, T. Is the acute NMDA receptor hypofunction a valid model of schizophrenia? *Schizophr. Bull.* **2012**, *38* (1), 9−14.

(10) Anastasio, N. C.; Xia, Y.; O'Connor, Z. R.; Johnson, K. M. Differential role of *N*-methyl-D-aspartate receptor subunits 2A and 2B in mediating phencyclidine-induced perinatal neuronal apoptosis and behavioral deficits. *Neuroscience* **2009**, *163* (4), 1181−1191.

(11) Jenkins, T. A.; Harte, M. K.; Reynolds, G. P. Effect of subchronic phencyclidine administration on sucrose preference and hippocampal parvalbumin immunoreactivity in the rat. *Neurosci. Lett.* **2010**, *471* (3), 144−157.

(12) Palsson, E.; Lowry, J.; Klamer, D. Information processing deficits and nitric oxide signalling in the phencyclidine model of schizophrenia. *Psychopharmacology* **2010**, *212* (4), 643−651.

(13) Mitchell, A. J.; Dinan, T. G. Schizophrenia: a multisystem disease? *J. Psychopharmacol.* **2010**, *24* (4 Suppl), 5−7.

(14) Domenici, E.; Wille, D. R.; Tozzi, F.; Prokopenko, I.; Miller, S.; McKeown, A.; Brittain, C.; Rujescu, D.; Giegling, I.; Turck, C. W.; Holsboer, F.; Bullmore, E. T.; Middleton, L.; Merlo-Pich, E.; Alexander, R. C.; Muglia, P. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. *PLoS One* **2010**, *5* (2), e9166.

(15) Schwarz, E.; Guest, P. C.; Rahmoune, H.; Harris, L. W.; Wang, L.; Leweke, F. M.; Rothermundt, M.; Bogerts, B.; Koethe, D.; Kranaster, L.; Ohrmann, P.; Suslow, T.; McAllister, G.; Spain, M.; Barnes, A.; van Beveren, N. J.; Baron-Cohen, S.; Steiner, J.; Torrey, F. E.; Yolken, R. H.; Bahn, S. Identification of a biological signature for schizophrenia in serum. *Mol. Psychiatry* **2012**, *17*, 494−502.

(16) Guest, P. C.; Wang, L.; Harris, L. W.; Burling, K.; Levin, Y.; Ernst, A.; Wayland, M. T.; Umrania, Y.; Herberth, M.; Koethe, D.; van Beveren, J. M.; Rothermundt, M.; McAllister, G.; Leweke, F. M.; Steiner, J.; Bahn, S. Increased levels of circulating insulin-related peptides in first-onset, antipsychotic naive schizophrenia patients. *Mol. Psychiatry* **2010**, *15* (2), 118−119.

(17) Holmes, E.; Tsang, T. M.; Huang, J. T.; Leweke, F. M.; Koethe, D.; Gerth, C. W.; Nolden, B. M.; Gross, S.; Schreiber, D.; Nicholson, J. K.; Bahn, S. Metabolic profiling of CSF: evidence that early intervention may impact on disease progression and outcome in schizophrenia. *PLoS Med.* **2006**, *3* (8), e327.

(18) Spelman, L. M.; Walsh, P. I.; Sharifi, N.; Collins, P.; Thakore, J. H. Impaired glucose tolerance in first-episode drug-naive patients with schizophrenia. *Diabetic Med.* **2007**, *24* (5), 481−485.

(19) Phillips, M.; Wang, C.; Johnson, K. M. Pharmacological characterization of locomotor sensitization induced by chronic phencyclidine administration. *J. Pharmacol. Exp. Ther.* **2001**, *296* (3), 905−13.

(20) Kalinichev, M.; Robbins, M. J.; Hartfield, E. M.; Maycox, P. R.; Moore, S. H.; Savage, K. M.; Austin, N. E.; Jones, D. N. Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague−Dawley rats: a locomotor activity and gene induction study. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2008**, *32* (2), 414−22.

(21) Schwarz, E.; Izmailov, R.; Spain, M.; Barnes, A.; Mapes, J. P.; Guest, P. C.; Rahmoune, H.; Pietsch, S.; Leweke, F. M.; Rothermundt, M.; Steiner, J.; Koethe, D.; Kranaster, L.; Ohrmann, P.; Suslow, T.; Levin, Y.; Bogerts, B.; van Beveren, N. J.; McAllister, G.; Weber, N.; Niebuhr, D.; Cowan, D.; Yolken, R. H.; Bahn, S. Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. *Biomarker Insights* **2010**, *5*, 39−47.

(22) Cheng, T. M.; Lu, Y. E.; Guest, P. C.; Rahmoune, H.; Harris, L. W.; Wang, L.; Ma, D.; Stelzhammer, V.; Umrania, Y.; Wayland, M. T.; Lio, P.; Bahn, S. Identification of targeted analyte clusters for studies of schizophrenia. *Mol. Cell. Proteomics* **2010**, *9* (3), 510−522.

(23) Martins-de-Souza, D.; Menezes de Oliveira, B.; dos Santos Farias, A.; Horiuchi, R. S.; Crepaldi Domingues, C.; de Paula, E.; Marangoni, S.; Gattaz, W. F.; Dias-Neto, E.; Camillo Novello, J. The use of ASB-14 in combination with CHAPS is the best for solubilization of human brain proteins for two-dimensional gel electrophoresis. *Briefings Funct. Genomics Proteomics* **2007**, *6* (1), 70−75.

(24) Krishnamurthy, D.; Levin, Y.; Harris, L. W.; Umrania, Y.; Bahn, S.; Guest, P. C. Analysis of the human pituitary proteome by data independent label-free liquid chromatography tandem mass spectrometry. *Proteomics* **2011**, *11* (3), 495−500.

(25) Lan, M. J.; McLoughlin, G. A.; Griffin, J. L.; Tsang, T. M.; Huang, J. T.; Yuan, P.; Manji, H.; Holmes, E.; Bahn, S. Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. *Mol. Psychiatry* **2009**, *14* (3), 269−279.

(26) Krishnamurthy, D.; Harris, L. W.; Levin, Y.; Koutroukides, T. A.; Rahmoune, H.; Pietsch, S.; Vanattou-Saifoudine, N.; Leweke, F. M.; Guest, P. C.; Bahn, S. Metabolic, hormonal and stress-related molecular

changes in post-mortem pituitary glands from schizophrenia subjects. *World J. Biol. Psychiatry* **2012**, in press.
(27) Chan, M. K.; Tsang, T. M.; Harris, L. W.; Guest, P. C.; Holmes, E.; Bahn, S. Evidence for disease and antipsychotic medication effects in post-mortem brain from schizophrenia patients. *Mol. Psychiatry* **2011**, *16* (12), 1189−1202.
(28) Sarnyai, Z.; Alsaif, M.; Bahn, S.; Ernst, A.; Guest, P. C.; Hradetzky, E.; Kluge, W.; Stelzhammer, V.; Wesseling, H. Behavioral and molecular biomarkers in translational animal models for neuropsychiatric disorders. *Int. Rev. Neurobiol.* **2011**, *101*, 203−38.
(29) Schubert, K. O.; Focking, M.; Prehn, J. H.; Cotter, D. R. Hypothesis review: are clathrin-mediated endocytosis and clathrin-dependent membrane and protein trafficking core pathophysiological processes in schizophrenia and bipolar disorder? *Mol Psychiatry* **2011**, DOI: 10.1038/mp.2011.123.
(30) Jeans, A.; Malins, R.; Padamsey, Z.; Reinhart, M.; Emptage, N. Increased expression of dysbindin-1A leads to a selective deficit in NMDA receptor signaling in the hippocampus. *Neuropharmacology* **2011**, *61* (8), 1345−1353.
(31) Blondeau, F.; Ritter, B.; Allaire, P. D.; Wasiak, S.; Girard, M.; Hussain, N. K.; Angers, A.; Legendre-Guillemin, V.; Roy, L.; Boismenu, D.; Kearney, R. E.; Bell, A. W.; Bergeron, J. J.; McPherson, P. S. Tandem MS analysis of brain clathrin-coated vesicles reveals their critical involvement in synaptic vesicle recycling. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101* (11), 3833−3838.
(32) Karlsson, R. M.; Tanaka, K.; Heilig, M.; Holmes, A. Loss of glial glutamate and aspartate transporter (excitatory amino acid transporter 1) causes locomotor hyperactivity and exaggerated responses to psychotomimetics: rescue by haloperidol and metabotropic glutamate 2/3 agonist. *Biol. Psychiatry* **2008**, *64* (9), 810−4.
(33) Rao, J. S.; Kellom, M.; Reese, E. A.; Rapoport, S. I.; Kim, H. W. Dysregulated glutamate and dopamine transporters in postmortem frontal cortex from bipolar and schizophrenic patients. *J. Affective Disord.* **2012**, *136* (1−2), 63−71.
(34) Bauer, D.; Haroutunian, V.; Meador-Woodruff, J. H.; McCullumsmith, R. E. Abnormal glycosylation of EAAT1 and EAAT2 in prefrontal cortex of elderly patients with schizophrenia. *Schizophr. Res.* **2010**, *117* (1), 92−98.
(35) Huerta, I.; McCullumsmith, R. E.; Haroutunian, V.; Gimenez-Amaya, J. M.; Meador-Woodruff, J. H. Expression of excitatory amino acid transporter interacting protein transcripts in the thalamus in schizophrenia. *Synapse* **2006**, *59* (7), 394−402.
(36) Ma, D.; Chan, M. K.; Lockstone, H. E.; Pietsch, S. R.; Jones, D. N.; Cilia, J.; Hill, M. D.; Robbins, M. J.; Benzel, I. M.; Umrania, Y.; Guest, P. C.; Levin, Y.; Maycox, P. R.; Bahn, S. Antipsychotic treatment alters protein expression associated with presynaptic function and nervous system development in rat frontal cortex. *J. Proteome Res.* **2009**, *8* (7), 3284−3297.
(37) Chana, G.; Lucero, G.; Salaria, S.; Lozach, J.; Du, P.; Woelk, C.; Everall, I. Upregulation of NRG-1 and VAMP-1 in human brain aggregates exposed to clozapine. *Schizophr. Res.* **2009**, *113* (2−3), 273−276.
(38) Hashimoto, K.; Engberg, G.; Shimizu, E.; Nordin, C.; Lindstrom, L. H.; Iyo, M. Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. *BMC Psychiatry* **2005**, *5*, 6.
(39) Heresco-Levy, U.; Ermilov, M.; Lichtenberg, P.; Bar, G.; Javitt, D. C. High-dose glycine added to olanzapine and risperidone for the treatment of schizophrenia. *Biol. Psychiatry* **2004**, *55* (2), 165−171.
(40) Tsai, G. E.; Yang, P.; Chang, Y. C.; Chong, M. Y. D-Alanine added to antipsychotics for the treatment of schizophrenia. *Biol. Psychiatry* **2006**, *59* (3), 230−234.
(41) Lutkenhoff, E. S.; van Erp, T. G.; Thomas, M. A.; Therman, S.; Manninen, M.; Huttunen, M. O.; Kaprio, J.; Lonnqvist, J.; O'Neill, J.; Cannon, T. D. Proton MRS in twin pairs discordant for schizophrenia. *Mol. Psychiatry* **2010**, *15* (3), 308−18.
(42) Seese, R. R.; O'Neill, J.; Hudkins, M.; Siddarth, P.; Levitt, J.; Tseng, B.; Wu, K. N.; Caplan, R. Proton magnetic resonance spectroscopy and thought disorder in childhood schizophrenia. *Schizophr. Res.* **2011**, *133* (1−3), 82−90.
(43) Shapiro, J.; Belmaker, R. H.; Biegon, A.; Seker, A.; Agam, G. Scyllo-inositol in post-mortem brain of bipolar, unipolar and schizophrenic patients. *J. Neural Transm.* **2000**, *107* (5), 603−607.
(44) Okulski, P.; Jay, T. M.; Jaworski, J.; Duniec, K.; Dzwonek, J.; Konopacki, F. A.; Wilczynski, G. M.; Sanchez-Capelo, A.; Mallet, J.; Kaczmarek, L. TIMP-1 abolishes MMP-9-dependent long-lasting long-term potentiation in the prefrontal cortex. *Biol. Psychiatry* **2007**, *62* (4), 359−62.
(45) Chaillan, F. A.; Rivera, S.; Marchetti, E.; Jourquin, J.; Werb, Z.; Soloway, P. D.; Khrestchatisky, M.; Roman, F. S. Involvement of tissue inhibition of metalloproteinases-1 in learning and memory in mice. *Behav. Brain Res.* **2006**, *173* (2), 191−8.
(46) Gawryluk, J. W.; Wang, J. F.; Andreazza, A. C.; Shao, L.; Yatham, L. N.; Young, L. T. Prefrontal cortex glutathione S-transferase levels in patients with bipolar disorder, major depression and schizophrenia. *Int. J. Neuropsychopharmacol.* **2011**, *14* (8), 1069−1074.
(47) Cui, J.; Shao, L.; Young, L. T.; Wang, J. F. Role of glutathione in neuroprotective effects of mood stabilizing drugs lithium and valproate. *Neuroscience* **2007**, *144* (4), 1447−1453.
(48) Yao, J. K.; Reddy, R. Oxidative stress in schizophrenia: pathogenetic and therapeutic implications. *Antioxid. Redox Signaling* **2011**, *15* (7), 1999−2002.
(49) Jones, K. Insulin coma therapy in schizophrenia. *J. R. Soc. Med.* **2000**, *93* (3), 147−149.
(50) Chintoh, A. F.; Mann, S. W.; Lam, L.; Giacca, A.; Fletcher, P.; Nobrega, J.; Remington, G. Insulin resistance and secretion in vivo: effects of different antipsychotics in an animal model. *Schizophr. Res.* **2009**, *108* (1−3), 127−133.
(51) Fernandez-Egea, E.; Miller, B.; Garcia-Rizo, C.; Bernardo, M.; Kirkpatrick, B. Metabolic effects of olanzapine in patients with newly diagnosed psychosis. *J. Clin. Psychopharmacol.* **2011**, *31* (2), 154−159.
(52) Lozovsky, D.; Saller, C. F.; Bayorh, M. A.; Chiueh, C. C.; Rice, K. C.; Burke, T. R., Jr.; Kopin, I. J. Effects of phencyclidine on rat prolactin, dopamine receptor and locomotor activity. *Life Sci.* **1983**, *32* (24), 2725−2731.
(53) Meltzer, H. Y.; Simonovic, M.; Gudelsky, G. A. Phencyclidine-induced inhibition of rat prolactin secretion: increased portal blood dopamine. *Eur. J. Pharmacol.* **1985**, *110* (1), 143−146.
(54) Cathcart, H. M.; Huang, R.; Lanham, I. S.; Corder, E. H.; Poduslo, S. E. Cystatin C as a risk factor for Alzheimer disease. *Neurology* **2005**, *64* (4), 755−757.
(55) Hansson, S. F.; Andreasson, U.; Wall, M.; Skoog, I.; Andreasen, N.; Wallin, A.; Zetterberg, H.; Blennow, K. Reduced levels of amyloid-beta-binding proteins in cerebrospinal fluid from Alzheimer's disease patients. *J. Alzheimers Dis.* **2009**, *16* (2), 389−397.
(56) Pechnick, R. N.; George, R.; Poland, R. E. Naloxone does not antagonize PCP-induced stimulation of the pituitary-adrenal axis in the rat. *Life Sci.* **1989**, *44* (2), 143−147.
(57) Zinger, M.; McFarland, M.; Ben-Jonathan, N. Prolactin expression and secretion by human breast glandular and adipose tissue explants. *J. Clin. Endocrinol. Metab.* **2003**, *88* (2), 689−696.
(58) Pilo, B.; Mehan, S. P. Effect of ACTH on glucose uptake by hepatic cells and its effect on hepatic enzymes in presence of insulin and acetylcholine. *Indian J. Physiol. Pharmacol.* **1986**, *30* (2), 155−160.
(59) Steppan, C. M.; Bailey, S. T.; Bhat, S.; Brown, E. J.; Banerjee, R. R.; Wright, C. M.; Patel, H. R.; Ahima, R. S.; Lazar, M. A. The hormone resistin links obesity to diabetes. *Nature* **2001**, *409* (6818), 307−312.
(60) Lazar, M. A. Resistin- and obesity-associated metabolic diseases. *Horm. Metab. Res.* **2007**, *39* (10), 710−716.
(61) Owecki, M.; Miczke, A.; Nikisch, E.; Pupek-Musialik, D.; Sowinski, J. Serum resistin concentrations are higher in human obesity but independent from insulin resistance. *Exp. Clin. Endocrinol. Diabetes* **2011**, *119* (2), 117−121.
(62) Bartoli, E.; Fra, G. P.; Carnevale Schianca, G. P. The oral glucose tolerance test (OGTT) revisited. *Eur. J. Intern. Med.* **2011**, *22* (1), 8−12.

(63) Ryan, M. C.; Thakore, J. H. Physical consequences of schizophrenia and its treatment: the metabolic syndrome. *Life Sci.* **2002**, *71* (3), 239−257.
(64) Meyer, J. M.; Davis, V. G.; McEvoy, J. P.; Goff, D. C.; Nasrallah, H. A.; Davis, S. M.; Daumit, G. L.; Hsiao, J.; Swartz, M. S.; Stroup, T. S.; Lieberman, J. A. Impact of antipsychotic treatment on nonfasting triglycerides in the CATIE Schizophrenia Trial phase 1. *Schizophr. Res.* **2008**, *103* (1−3), 104−109.
(65) Dasgupta, A.; Singh, O. P.; Rout, J. K.; Saha, T.; Mandal, S. Insulin resistance and metabolic profile in antipsychotic naive schizophrenia patients. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2010**, *34* (7), 1202−1207.
(66) Sharma, A. N.; Elased, K. M.; Garrett, T. L.; Lucot, J. B. Neurobehavioral deficits in db/db diabetic mice. *Physiol. Behav.* **2010**, *101* (3), 381−388.
(67) Sharma, A. N.; Elased, K. M.; Lucot, J. B. Rosiglitazone treatment reversed depression- but not psychosis-like behavior of db/db diabetic mice. *J. Psychopharmacol.* **2012**, in press.
(68) Guest, P. C.; Schwarz, E.; Krishnamurthy, D.; Harris, L. W.; Leweke, F. M.; Rothermundt, M.; van Beveren, N. J.; Spain, M.; Barnes, A.; Steiner, J.; Rahmoune, H.; Bahn, S. Altered levels of circulating insulin and other neuroendocrine hormones associated with the onset of schizophrenia. *Psychoneuroendocrinology* **2011**, *36* (7), 1092−1096.
(69) Cardoso, S.; Carvalho, C.; Santos, R.; Correia, S.; Santos, M. S.; Seica, R.; Oliveira, C. R.; Moreira, P. I. Impact of STZ-induced hyperglycemia and insulin-induced hypoglycemia in plasma amino acids and cortical synaptosomal neurotransmitters. *Synapse* **2011**, *65* (6), 457−466.
(70) Halim, N. D.; Lipska, B. K.; Hyde, T. M.; Deep-Soboslay, A.; Saylor, E. M.; Herman, M. M.; Thakar, J.; Verma, A.; Kleinman, J. E. Increased lactate levels and reduced pH in postmortem brains of schizophrenics: medication confounds. *J. Neurosci. Methods* **2008**, *169* (1), 208−13.
(71) Vawter, M. P.; Tomita, H.; Meng, F.; Bolstad, B.; Li, J.; Evans, S.; Choudary, P.; Atz, M.; Shao, L.; Neal, C.; Walsh, D. M.; Burmeister, M.; Speed, T.; Myers, R.; Jones, E. G.; Watson, S. J.; Akil, H.; Bunney, W. E. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. *Mol. Psychiatry* **2006**, *11* (7), 615, 663−679.
(72) Schwarz, E.; Izmailov, R.; Spain, M.; Barnes, A.; Mapes, J. P.; Guest, P. C.; Rahmoune, H.; Pietsch, S.; Leweke, F. M.; Rothermundt, M.; Steiner, J.; Koethe, D.; Kranaster, L.; Ohrmann, P.; Suslow, T.; Levin, Y.; Bogerts, B.; van Beveren, N. J.; McAllister, G.; Weber, N.; Niebuhr, D.; Cowan, D.; Yolken, R. H.; Bahn, S. Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. *Biomarker Insights* **2010**, *5*, 39−47.
(73) Cloarec, O.; Dumas, M. E.; Craig, A.; Barton, R. H.; Trygg, J.; Hudson, J.; Blancher, C.; Gauguier, D.; Lindon, J. C.; Holmes, E.; Nicholson, J. Statistical total correlation spectroscopy: an exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. *Anal. Chem.* **2005**, *77* (5), 1282−1289.