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Changes in gene expression after phencyclidine administration in developing rats: a potential animal model for schizophrenia

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Abstract

Repeated administration of phencyclidine (PCP), an *N*-methyl-D-aspartate (NMDA) receptor antagonist, during development, may result in neuronal damage that leads to behavioral deficits in adulthood. The present study examined the potential neurotoxic effects of PCP exposure (10 mg/kg) in rats on postnatal days (PNDs) 7, 9 and 11 and the possible underlying mechanism(s) for neurotoxicity. Brain tissue was harvested for RNA extraction and morphological assessments. RNA was collected from the frontal cortex for DNA microarray analysis and quantitative RT-PCR. Gene expression profiling was determined using Illumina Rat Ref-12 Expression BeadChips containing 22,226 probes. Based on criteria of a fold-change greater than 1.4 and a *P*-value less than 0.05, 19 genes including NMDAR1 (*N*-methyl-D-aspartate receptor) and four pro-apoptotic genes were up-regulated, and 25 genes including four anti-apoptotic genes were down-regulated, in the PCP-treated group. In addition, the schizophrenia-relevant genes, *Bdnf* (Brain-derived neurotrophic factor) and *Bhlhb2* (basic helix-loop-helix domain containing, class B, 2), were significantly different between the PCP and the control groups. Quantitative RT-PCR confirmed the microarray results. Elevated neuronal cell death was further confirmed using Fluoro-Jade C

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staining. These findings support the hypothesis that neurodegeneration caused by PCP occurs, at least in part, through the up-regulation of NMDA receptors, which makes neurons possessing these receptors more vulnerable to endogenous glutamate. The changes in schizophrenia-relevant genes after repeated PCP exposure during development may provide important information concerning the validation of an animal model for this disorder.

Keywords

Apoptosis; Neuronal development; DNA microarray; Gene expression; Phencyclidine (PCP); Schizophrenia

1. Introduction

Schizophrenia is a chronic, severe, and disabling brain disorder that affects about 1% of the world's population (Bromet and Fennig, 1999). It is characterized by abnormalities in the perception or expression of reality. Schizophrenia affects multiple cognitive-behavioral domains, comprising positive symptoms (e.g., delusion, hallucination, and paranoia), negative symptoms (e.g., loss of motivation, affective blunting and social withdrawal), and cognitive symptoms. Despite intensive studies, its molecular etiology remains enigmatic. Because many drugs that ameliorate psychotic symptoms in patients with schizophrenia are dopamine receptor blockers, much attention has been devoted to the dopamine hyperactivity hypothesis. However, it is difficult to explain the disease solely in terms of an abnormally overactive dopaminergic transmitter system (Olney and Farber, 1995). Several studies on families, twins, and adoptions suggest the importance of genetic factors in etiology; while clinical studies, advanced imaging techniques such as magnetic resonance imaging (MRI), and novel neuroanatomical markers have provided evidence that schizophrenia is a neurodevelopmental disorder (Sawa and Snyder, 2002). Some investigators have suggested that NMDA receptor hypofunction may underlie certain features of schizophrenia (Olney and Farber, 1995). In fact, early in 1980, Kim et al. (1980) first proposed that decreased glutamatergic activity may be involved in the etiology of schizophrenia. The accretion of evidence in support of the hypothesis that hypofunction of NMDA receptors contributes to the symptoms of schizophrenia, has provided the first compelling alternative to the dopamine hypothesis, even a more important role in the endophenotype of schizophrenia than dopamine (Coyle, 2006). In addition to the abnormalities in GABAergic neurotransmission in schizophrenia (Reynolds and Harte, 2007), the involvement of the phosphatidylinositol 3-kinase (PI3K) pathway, has also been suggested to contribute to schizophrenia (Brazil and Hemmings, 2001; Kalkman, 2006).

To better understand the mechanisms of schizophrenia, animal models have been developed, although none of the current animal models can serve as a complete animal equivalent to the human disorder. For example, an insurmountable obstacle of animal models is the inability to exhibit certain schizophrenia deficits, such as verbal behavior, making it impossible to produce a comprehensive animal model of schizophrenia. The *N*-methyl-D-aspartate (NMDA) receptor antagonists, including phencyclidine (PCP) and ketamine, seem to be capable of inducing both positive and negative symptoms of schizophrenia, including

cognitive dysfunction in normal patients (Javitt and Zukin, 1991; Snyder, 1980; Tamminga, 1998); these drugs also profoundly exacerbate both positive and negative symptoms in schizophrenia patients (Lahti et al., 1995; Malhotra et al., 1997). Because of the more comprehensive psychopathology induced by PCP, many researchers have studied the effects of PCP in humans and animals to gain insights into the mechanisms of schizophrenia. Two separate groups (Jentsch et al., 1997; Johnson et al., 1998) reported that repeated PCP administration in adult rats produced behavioral, cellular and biochemical deficits sensitive to antipsychotics. Wang et al. (2001) demonstrated that perinatal PCP administration produces behavioral deficits in puberty. However, the behavioral deficits and underlying neuronal plasticity observed in adult animals may not be the same as the changes in the developing brain initiated by a perinatal insult. It has been demonstrated that some cases of schizophrenia may be the result of an insult during the prenatal or perinatal period (Benes et al., 1991; Murray et al., 1992; Pilowsky et al., 1993), though the functional consequences of the insult are not apparent until after puberty, when the affected neural networks reach maturity (Weinberger, 1987). Since it is a popular schizophrenia animal model, PCP-treated animals have been used to test some specific mechanistic hypotheses. It is evident that no single molecular event could be completely explanatory of the pathophysiology of schizophrenia. Therefore, a complete study of gene expression changes in this model would be helpful. In the present study, an animal model was developed using perinatal rats and repeated PCP administration to explore the brain gene expression profile and provide further evidence to validate an animal model of schizophrenia.

2. Material and methods

2.1. Animal treatment

Sprague–Dawley rat pups (both male and female) were used and randomly divided into either a PCP-treated or control (saline) group. All animal procedures were approved by the Institutional Animal Care and Use Committee of the National Center for Toxicological Research (NCTR)/U.S. Food and Drug Administration (FDA) and conducted in full accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. PCP was acquired from the National Institute on Drug Abuse (Rockville, MD, USA) and was dissolved in 0.9% NaCl (saline vehicle).

Similar to previous studies (Wang et al., 2001, 2003), rat pups received 10 mg/kg PCP, s.c. ($n = 10$, 5 animals for the histochemical study and 5 animals for DNA microarray analyses) or saline, s.c. ($n = 10$, 5 animals for the histochemical study and 5 animals for DNA microarray analyses) on postnatal days (PNDs) 7, 9 and 11. The animals were returned to their dams between injections. A previous study in the rat (James and Schnoll, 1976) has shown that 24 h after PCP treatment the concentrations of PCP in the blood and brain are nearly undetectable. Therefore, on PND 12, 24 h after the final PCP administration, pups for microarray analyses were sacrificed and their brains were collected for RNA isolation. Meanwhile, pups for the histochemical study received a transaortic perfusion of 0.9% saline and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2).

2.2. RNA extraction from brains

Tissues from the frontal cortex were collected for RNA extraction. Total RNA was isolated with RNeasy® Lipid Tissue Mini Kits (Qiagen Inc., Valencia, CA). The extracted RNA was assessed spectrophotometrically by measuring the optical density at 260 nm. RNA purity and quality were evaluated using RNA 6000 LabChip Kits and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). High quality RNA with RNA integrity numbers (RINs) greater than 8.5 was used for the microarray experiments and TaqMan gene expression assays.

2.3. Microarray analysis

To assess gene expression profiles in PCP-treated rat brains, microarray techniques were employed to examine the gene expression patterns in the PND 12 rat brains. Gene expression profiling was performed using the Illumina Rat Ref-12 Expression BeadChip platform containing 22,226 probes (Illumina Inc., San Diego, CA). Gene expression data from the Illumina Rat Ref-12 arrays were analyzed using ArrayTrack™, a software system developed at the NCTR/FDA for the management, analysis, visualization and interpretation of microarray data (<http://www.fda.gov/nctr/science/centers/toxicoinformatics/ArrayTrack/>). Microarray data from five treated animals were compared to those from four control animals (one array from the control group was not used in the data analysis because of poor array quality). The differentially expressed genes (DEGs) were selected using criteria of a *P*-value less than 0.05 and a fold-change greater than 1.4 (up or down). This straightforward gene selection method, which combines a *P*-value cutoff (e.g. 0.05) and a fold-change ranking, has been extensively demonstrated to result in a higher level of concordance among lists of DEGs when the same set of samples were reanalyzed with different microarray platforms or in different laboratories with the same microarray platform (Guo et al., 2006; Shi et al., 2006). The use of a *P* < 0.05 criterion controls the chance of false discovery (*i.e.* calling a gene as differentially expressed when it is in fact not), whereas ranking the remaining genes that meet the *P* < 0.05 criterion by the fold-change criterion allows for genes with a larger magnitude of differential expression to be reproducibly identified as truly differentially expressed, thus balancing the sensitivity, specificity, and reproducibility of DEGs from a microarray gene expression study (Shi et al., 2008). It should be noted that the choice of a cutoff for fold-change, like for any other statistical measures, is arbitrary. The choice can be influenced by many factors such as the degree of the inherent differences between the groups of study samples and the number of DEGs that the researchers are willing to consider. For studies with larger between-group differences, such as in comparing different tissue types, a more stringent fold-change cutoff may be applied, whereas for studies with smaller between-group differences, such as comparing the same brain tissue type before and after chemical treatment, a less stringent fold-change cutoff may be needed to make sure that some genes with biological significance and a statistically significant *P*-value are included. In this study, the combination of *P* < 0.05 and fold-change greater than 1.4 resulted in a reasonable number of genes selected as differentially expressed on which biological interpretation was focused. It is important to point out that some genes that do not meet these selection criteria may indeed be differentially expressed, but with different levels of confidence.

2.4. TaqMan gene expression assays

The expression levels of the following genes were measured by quantitative RT-PCR (Q-PCR) using TaqMan assays (Applied Biosystems, Foster City, CA). The Taq-Man probes included *Bdnf* (Brain-derived neurotrophic factor) (Rn01484924 m1), *Bhlhb2* (basic helix-loop-helix domain containing, class B, 2) (Rn00584155 m1), and *Grin1* (NR1) (Rn01436038 m1). Two genes, *Polr2a* (RNA polymerase II A) (Rn01752026 m1) and *Actb* (β -actin) (Rn00667869 m1), were used for endogenous controls.

Each assay was run in triplicate for each RNA sample. Total cDNA (20 ng) in a 20 μ l final volume was used for each assay. Assays were run with Universal Master Mix (2x) without AmpErase UNG on a Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA) using universal cycling conditions (10 min at 95 °C; then 15 s at 95 °C and 1 min at 60 °C for 40 cycles).

2.4.1. First strand cDNA synthesis—cDNA was prepared using a High-Capacity cDNA Archive Kit (Applied Biosystems). Briefly, total RNA (2 μ g) was reverse-transcribed in a final volume of 20 μ l with random primers at 25 °C for 10 min followed by 37 °C for 120 min according to the manufacturer's instructions.

2.4.2. Data normalization and analysis—Two endogenous control genes, *Actb* and *Polr2a*, were used for normalization. Each replicate cycle threshold (C_T) was normalized to the average C_T of the two endogenous controls on a per sample basis. The comparative C_T method was used to calculate relative quantitation of gene expression (Livak and Schmittgen, 2001). The following formula was used to calculate the relative amount of the transcripts in the PCP-treated samples (treated) and the saline-treated samples (control), and both were normalized to the endogenous controls. $C_T = C_T(\text{treated}) - C_T(\text{control})$.

C_T is the difference in C_T between the target gene and endogenous controls by subtracting the average C_T of controls. The fold-change for the PCP-treated sample relative to the control sample = 2^{-C_T} .

2.5. Fluoro-Jade C staining

Fluoro-Jade C is a specific stain for degenerating neurons in brain tissue (Schmued and Hopkins, 2000). Prior to staining, adjacent sections (10 μ m) were mounted onto positively charged slides. Slides were first immersed in a basic alcohol solution consisting of 1% sodium hydroxide in 80% ethanol for 5 min, then washed with 70% ethanol and distilled water, and incubated in 0.06% potassium permanganate solution for 10 min. Slides were subsequently transferred to a 0.0001% solution of Fluoro-Jade C (Histo-Chem Inc., Jefferson, AR) dissolved in 0.1% acetic acid for 25 min. The slides were rinsed with distilled water, air dried, cleared in xylene and cover-slipped with DPX nonfluorescent mounting media (Sigma, St. Louis, MO).

2.6. Statistical analysis

The data were analyzed by *t*-test. Quantitative analysis of Fluoro-Jade C staining was presented as the mean values $\pm$ S.E.M. The null hypothesis was rejected at a probability level of $P < 0.05$.

3. Results

3.1. Gene expression profile of microarray data

Hierarchical cluster analysis (HCA) (Fig. 1) and principal component analysis (PCA) (Fig. 2) were used to describe the gene expression profiles of control and PCP-treated samples. Two apparent clusters were revealed in both HCA and PCA with the samples separating well into control and treated groups. These results indicated clear effects of PCP in the treated samples. It should be noted that the data from a total of 22,226 probes (the entire data set), without any specific filtering, were utilized for the clustering analysis and principal component analysis.

3.2. PCP-induced changes in gene expression

A criterion of $P < 0.05$ derived from an appropriate t -test between the control and PCP-treated groups with various fold-changes was employed to generate a list of DEGs. There were 1703 DEGs at various fold changes with a $P < 0.05$. Combination of a fold-change criterion with a minimum P -value cutoff is a straightforward method to identify DEGs (Guo et al., 2006). Therefore, a fold-change greater than 1.4 and a $P < 0.05$ were arbitrarily selected for further analyses. Based on these criteria, 19 genes were up-regulated and 25 genes were down-regulated in the PCP-treated group. NMDA receptor NR1 subunit mRNA was significantly up-regulated. Four up-regulated genes are thought to positively regulate apoptosis and 4 down-regulated genes are thought to negatively regulate apoptosis, suggesting neurodegeneration plays a critical role in the PCP-treated rats, and up-regulation of the NMDA receptor may be a cause of the neurodegeneration observed in rat brains. In addition, schizophrenia-relevant genes, Bdnf (Brain-derived neurotrophic factor) and Bhlhb2 (basic helix-loop-helix domain containing, class B, 2), were significantly different between the PCP-treated group and the control group. A summary of these DEGs is shown in Table 1.

3.3. Q-PCR validation of the microarray results

Q-PCR assays (TaqMan assays) were used to confirm the results of the gene expression changes measured using microarrays, with particular focus on schizophrenia-relevant genes, such as Bdnf and Bhlhb2. The Q-PCR data correlated well with the microarray results. The Q-PCR assay demonstrated that the expression of Bdnf and Bhlhb2 were significantly down-regulated and up-regulated, respectively. The expression patterns of changes were nearly identical using the two techniques (Table 2).

3.4. Morphological confirmation of PCP-induced neurodegeneration

Enhanced neuronal degeneration was apparent in the frontal cortex and cingulate cortex, as indicated by a remarkable increase in the number of Fluoro-Jade C-stained positive cells in PCP-exposed animals (PND 7 rat pups) compared with control (Fig. 3A). However, only a few Fluoro-Jade C positive cells were observed, and no significant effects were detected in some other major brain regions such as the striatum, hippocampus, thalamus and amygdala in rats repeatedly exposed to PCP compared to controls. Statistical analyses (t -test) demonstrated that repeated PCP exposure during development resulted in a significant

increase in Fluoro-Jade C-stained positive neurons in layers II and III of the frontal cortex (Fig. 3B) and cingulate cortex. Meanwhile, enhanced neuronal degeneration was not observed in the striatum, hippocampus, thalamus and amygdala compared to the controls (data not shown).

4. Discussion

In the current study, the decision to begin the administration of PCP to rats on PND 7 was based on data that demonstrate that the first 2 weeks of postnatal life in rats correspond to the synaptogenesis period, also known as the brain growth-spurt period (Tenkova et al., 2003). During this period of rapid synaptogenesis, exposure to viral or environmental insults increases the predisposition to developing schizophrenia in adulthood (Clancy et al., 2001). Ketamine, another NMDA receptor antagonist, is known to induce psychosis in an age-dependent manner, with susceptibility to these effects not occurring in humans until adolescence, and peak sensitivity occurring in early adulthood (Reich and Silvey, 1989). It has also been suggested that PCP-induced psychosis may have a similar age-dependency profile (Baldrige and Bessen, 1990; Welch and Correa, 1980). One possible explanation for this post-pubertal occurrence can be that other brain regions compensate for damage before puberty, but by adolescence the brain becomes developmentally committed to use the cortex for this activity (Marcotte et al., 2001).

Consistent with our previous histochemical data (Wang et al., 2000), repeated PCP administration (10 mg/kg; on PNDs 7, 9 and 11) resulted in a significant increase in the number of Fluoro-Jade C-stained positive neurons in layers II and III of the frontal cortex and cingulate cortex. Elevated neuronal cell death during the perinatal period could directly produce developmental brain deficits and disturbed neuronal cytoarchitecture and plasticity. It is quite possible that these architectural deficits during development might contribute, as the morphological bases, to the onset of schizophrenia-relevant symptoms (some positive and negative symptoms) in adulthood.

The present DNA microarray and Q-PCR data give a relatively complete picture of gene expression in the developing rat brain after repeated PCP exposure. The microarray data revealed that several genes which have a *P*-value less than 0.05 and a fold-change greater than 1.4 (up or down) in the PCP-treated group were apoptosis-related genes. Among them, four up-regulated genes (*Txnip*, *Dnmt3a*, *Tieg*, and *Ddit4*) were pro-apoptotic genes. *Txnip*, encoding thioredoxin interacting protein, which binds thioredoxin and inhibits its activity, promotes vulnerability to oxidative stress of neurons (Schulze et al., 2004; Yoshida et al., 2005). *Txnip* mRNA was significantly increased ($P = 0.00004$, fold-change = 2.4) in the PCP-treated rat pups. Papadia et al. (2008) confirmed NMDA receptor dependent *Txnip* regulation by western blot in rat cortical neurons. They also found that *Txnip* expression reduced the antioxidative effects of synaptic activity, which indicated that *Txnip* influences neuronal antioxidant defense, rendering neurons vulnerable to oxidative stress. The previous microarray study from our laboratory also showed significantly enhanced *Txnip* mRNA in perinatal rat brains using ketamine, another NMDA receptor antagonist (Shi et al., 2010). The microarray analyses also showed *Mt1a* (Metallothionein 1 A) mRNA was increased with a fold-change of 1.6 ($P = 0.0006$) after PCP administration. It is known that the

expression of Mt1a can be induced at the transcriptional level by a variety of stressors, such as reactive oxygen species (ROS) (Spee et al., 2005). Increased gene expression of Mt1a may indicate increased ROS generation in PCP-treated neurons. In fact, Wang et al. (2000) gave evidence supporting the role of ROS in PCP-treated cultured forebrain neurons.

In addition, repeated PCP exposure decreased the expression of four anti-apoptotic genes in comparison with controls (Pcp4, Igf2, Ptgs2, and Cited2). Of these genes, Cited2 mRNA was significantly decreased with a fold-change of 1.5 in PCP-treated rats. Barbera et al. (2002) have presented strong evidence that Cited2 has the capability of inhibiting neuronal apoptosis. Thus, the obvious neurodegeneration detected by Fluoro-Jade C staining in PCP-treated rats may be a consequence of the highly expressed pro-apoptotic genes and down-regulation of anti-apoptotic genes.

Our previous studies demonstrated that developmental PCP administration results in a significant up-regulation of NMDA receptor NR1 subunit mRNA in the rat frontal cortex (Wang et al., 2001). The DNA microarray and Q-PCR data presented here also highlight the potential role of NMDA receptor dysfunction in the development of schizophrenia. In the present study, microarray analyses demonstrated that *N*-methyl-D-aspartate-like receptor 1A (XM 576765.1) mRNA was highly expressed in repeated PCP-exposed rat pups compared with controls. The elevated expression level of NR1 was further confirmed using Q-PCR, with the TaqMan probes targeting gene *Grin1* (NR1) (Rn01436038 m1) in PCP-treated animals. These findings support our hypothesis that continually blocking NMDA receptors with NMDA receptor antagonists, such as PCP or ketamine, induces a compensatory up-regulation of NMDA receptors on the neurons. The up-regulation of NMDA receptors allows for the accumulation of toxic levels of intracellular free calcium. Consequently, neurons are more vulnerable to the excitotoxic effects of endogenous glutamate after NMDA receptor antagonist (e.g., PCP or ketamine) withdrawal (washout).

Schizophrenia patients have difficulty in filtering information from their surroundings and have deficits in sensorimotor gating as measured by prepulse inhibition (Braff et al., 1992). Wang et al. (2001) demonstrated that perinatal PCP administration caused reduced prepulse inhibition, enhanced locomotor activity, altered acquisition of a delayed spatial learning task and sensorimotor gating; all of which may be related to the behavioral changes observed in schizophrenia. Our current data suggest that elevated neuron loss during the perinatal period would be a critical contributor for the behavioral changes observed in the PCP model. Another important new finding in the present study is the detected changes in gene expression levels which are critical for the evaluation of schizophrenia models. BDNF is characterized as a member of the neurotrophins which modulate the strength of existing synaptic connections and play a role in forming new synaptic contacts (Chao, 2003; Katz and Shatz, 1996; Lu and Figurov, 1997; Thoenen, 1995). BDNF is also critical in central nervous system development, neuronal survival, and rapid signaling (Ghosh et al., 1994; Kafitz et al., 1999; Marini et al., 1998; Schwartz et al., 1997). BDNF was found to protect striatal neurons from oxidative stress and apoptosis induced by neurotoxic dopamine exposure (Petersen et al., 2001). Lindholm et al. (1996) reported that neurons in the hippocampus from BDNF knockout mice exhibited enhanced cell death compared with cells from the wild-type mice. In addition, exogenous BDNF increased the survival of the

hippocampal neurons lacking BDNF. A neurotrophin hypothesis of schizophrenia (Thome et al., 1998) has postulated that changes in the brain of schizophrenia patients is the consequence of disturbances of developing processes involving these molecules. This hypothesis is supported by studies that reduced levels of BDNF were observed in both schizophrenia patients and animal models (Ashe et al., 2002; Durany et al., 2001; Iritani et al., 2003; Lipska et al., 2001; Toyooka et al., 2002). In line with these findings, the microarray and Q-PCR data presented here show that *Bdnf* mRNA was significantly decreased in PCP-treated rat brains, providing further evidence to substantiate a PCP animal model of schizophrenia.

Another schizophrenia-relevant gene, *Bhlhb2*, is a member of the basic helix-loop-helix (BHLH) super family of transcription factors. The *Bhlhb2* is regulated by NGF (a member of the neurotrophin family), and glutamate receptors (Rossner et al., 1997), suggesting that BHLHB2 is involved in plastic responses in the brain (Jiang et al., 2008). Jiang et al. (2008) demonstrated that *Bhlhb2* was a modulator of *Bdnf* transcription and contributes to neuronal excitability; and *Bdnf* was negatively modulated by *Bhlhb2*. The microarray data show a significant increase of *Bhlhb2* with a fold-change of 1.4. Thus, increased BHLHB2 and decreased BDNF may play a role in neuronal apoptosis and neuronal excitability in repeated PCP-induced schizophrenia models during development.

In summary, a repeated PCP-treated perinatal animal model was utilized to evaluate specific causative or mechanistic hypotheses of schizophrenia. The DNA microarray and Q-PCR data highlight the potential role of NMDA receptor dysfunction in PCP-induced neurodegeneration during development. With increased pro-apoptotic genes and decreased anti-apoptotic genes, it was proposed that the PCP-induced neuronal cell death during development might be apoptotic in nature. Furthermore, repeated PCP administration during development could result in significant regulation in the expression levels of schizophrenia-relevant genes, suggesting that this animal model may provide more than one facet for future schizophrenia studies.

Taken together, increased expression of NMDA-receptor NR1 subunit and pro-apoptotic genes, decreased anti-apoptotic genes, as well as altered expression of *Bdnf* and *Bhlhb2* may influence each other and exacerbate neurodegeneration in repeated PCP-exposed developing brains, subsequently resulting in abnormal development of neurons and neuronal networks, which might be critical for schizophrenia symptoms in adulthood (see Fig. 4).

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Fig. 1. Hierarchical cluster analysis (HCA) of expression profiles for control and PCP-treated groups. The log₂ intensity of each gene was scaled by Z-score transformation, and then these values were hierarchically clustered using ward's distance metric. The intensity of the entire gene set was used; no specific cutoff was applied for the analysis. Each column represents the results from an individual animal. Samples are labeled according to the convention of treatment-animal ID. PCP: PCP treatment; CTR: vehicle control.

Fig. 2.

Principal component analysis (PCA) of gene expression profiles for the PCP-treated groups and controls. The intensity of the entire gene set was used; no specific cutoff was applied for the analysis. The autoscaled method was used for the PCA plot. The triangles and circles indicate controls and PCP treatment, respectively.

(A) Fluoro-Jade C Staining

(B)

Fig. 3.

Representative pictures of neuronal degeneration in PCP-treated rat pups. Increased numbers of Fluoro-Jade C-stained positive neuronal cells were observed in layers II and III of the frontal cortex in PCP-treated rat pups compared to controls. Data are presented as means $\pm$ S.E.M. * $P < 0.05$ was considered significant. Scale bars = 50 μ m.

Fig. 4.
Hypothesized mechanistic flow chart for a PCP-induced schizophrenia model.

Table 1

Differentially expressed genes in PCP-treated rat brains selected using the criteria of $P < 0.05$ and fold-change greater than 1.4.

Gene symbol	Locus link ID	Gene description	Fold-change	P value
LOC499094	499094	Predicted: similar to zinc finger protein 61	2.4	0.00013
Txnip	117514	Up-regulated by 1,25-dihydroxyvitamin D-3	2.4	0.00004
Nr1d1	252917	Nuclear receptor subfamily 1, group D, member 1	2.1	0.00003
Rnf138	94196	Ring finger protein 138	1.9	0.02349
LOC499772	499772	Predicted: similar to immediate early response 5-like	1.8	0.00004
Mt1a	24567	Metallothionein	1.6	0.00055
Lcn2	170496	Lipocalin 2	1.6	0.00347
Dnmt3a	444984	Predicted: DNA methyltransferase 3A	1.6	0.00745
Ras11b	305302	RAS-like family 11 member B	1.5	0.00005
Tieg	81813	TGFB inducible early growth response	1.5	0.00022
Ddit4	140942	DNA-damage-inducible transcript 4	1.5	0.00859
Bhlhb2	79431	Basic helix-loop-helix domain containing, class B2	1.4	0.00011
Rap2ip	303569	Rap2 interacting protein	1.4	0.01632
Mta1	64520	Metastasis associated 1	1.4	0.00210
Clta	83800	Clathrin, light polypeptide (Lca)	1.4	0.01587
Nfkbia	25493	Predicted: nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	1.4	0.00055
Rnf7 predicted	300948	Predicted: ring finger protein 7 (predicted)	1.4	0.00262
LOC501354	501354	Predicted: similar to glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A	1.4	0.00409
Gsn	296654	Gelsolin	1.4	0.00794
LOC499103	499103	Predicted: similar to RIKEN cDNA A830041P22 gene	-1.4	0.00964
Mycn	298894	Predicted: v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	-1.4	0.00035
LOC501344	501344	Predicted: hypothetical gene supported by BC082068	-1.4	0.00001
Dusp6	116663	Dual specificity phosphatase 6	-1.4	0.00198
Slc25a3	245959	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 3	-1.4	0.00953
LOC366673	366673	Predicted: similar to vesicle transport through interaction with t-SNAREs 1B homolog	-1.4	0.00174
Dstn predicted	296197	Predicted: destrin (predicted)	-1.4	0.00402
Pcp4	25510	Purkinje cell protein 4	-1.4	0.01444
Igf2	24483	Insulin-like growth factor 2	-1.4	0.00338
Myc	24577	Myelocytomatosis viral oncogene homolog (avian)	-1.4	0.00372
Ptgs2	29527	Prostaglandin-endoperoxide synthase 2	-1.4	0.00018
Lanc12 predicted	297148	Predicted: LanC (bacterial lantibiotic synthetase component C)-like 2 (predicted)	-1.5	0.01472
Cited2	114490	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	-1.5	0.00239
Exosc3 predicted	313243	Predicted: exosome component 3 (predicted)	-1.5	0.00186
Sstr1	25033	Predicted: somatostatin receptor 1	-1.5	0.01077

Gene symbol	Locus link ID	Gene description	Fold-change	P value
Plekhf2 predicted	362484	Predicted: pleckstrin homology domain containing, family F (with FYVE domain) member 2 (predicted)	-1.5	0.00736
G0s2	289388	G0/G1 switch gene 2	-1.5	0.00004
Arc	54323	Activity regulated cytoskeletal-associated protein	-1.5	0.00178
Psm6	29673	Proteasome (prosome, macropain) subunit, alpha type 6	-1.5	0.04129
Ankrd15 predicted	309429	Predicted: ankyrin repeat domain 15 (predicted)	-1.6	0.03166
Tnfrsf11b	25341	Tumor necrosis factor receptor super family, member 11b (osteoprotegerin)	-1.7	0.00001
Clk1 predicted	301434	Predicted: CDC-like kinase 1 (predicted)	-1.8	0.00059
RGD1303272	362134	Similar to RIKEN cDNA 2010311D03	-1.9	0.02512

Table 2

Selective confirmation of schizophrenia-relevant gene expression by Q-PCR.

Gene symbols	Fold-change (Q-PCR)	Fold-change (microarray)
Bdnf	1.41 *	1.31 *
Bhlhb2	1.48 *	1.45 *

* $P < 0.05$ as compared to the control.