**Title**: Synapse-Associated Protein 90/Postsynaptic Density-95-Associated Protein (SAPAP) is Expressed Differentially in Phencyclidine-Treated Rats and is Increased in the Nucleus Accumbens of Patients with Schizophrenia

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Synapse-associated protein 90/postsynaptic density-95-associated protein (SAPAP) is expressed differentially in phencyclidine-treated rats and is increased in the nucleus accumbens of patients with schizophrenia

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Running title: SAPAP and schizophrenia

Abstract
Phencyclidine (PCP) induces a psychotomimetic state that closely resembles schizophrenia. Therefore, PCP-treated animals can provide a model for schizophrenia. Using differential display, we identified a gene regulated by the delayed action of PCP in rat nucleus accumbens (NAc). Sequence analysis showed that the cDNA clone obtained was identical to rat synapse-associated protein 90/postsynaptic density-95-associated protein 1 (SAPAP1). Quantitative reverse transcriptase (RT)-PCR analysis showed that SAPAP1 mRNA had increased significantly in rat NAc ($P<0.0001$) and hippocampus ($P<0.01$) 24 hours after a PCP (10 mg/kg) injection as compared to the controls. Immunoquantification using an anti-SAPAP1 antibody indicated that immunoreactivity for SAPAP1 increased significantly ($P<0.05$) in the NAc of unmedicated patients with schizophrenia, as compared to the control subjects and medicated patients with schizophrenia. Our findings support the hypothesis that there is abnormal glutamatergic neurotransmission in schizophrenia and show evidence of abnormalities in the intracellular signal transduction via $N$-methyl-$d$-aspartate (NMDA) receptors.

**Key Words:** Phencyclidine (PCP); Differential display; Glutamate; NMDA; Schizophrenia; Brain;

**INTRODUCTION**
There has been considerable interest in the pathophysiological role of glutamate in schizophrenia (Tamminga 1998; Meador-Woodruff and Healy 2000; Goff and Coyle 2001). Strong evidence that supports an association between glutamatergic dysregulation and schizophrenia has come from pharmacological studies showing that N-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine (PCP) and ketamine, induce schizophrenia-like symptoms in normal volunteers and exacerbate psychotic symptoms in schizophrenic patients (Luby et al. 1962; Javitt and Zukin 1991; Krystal et al. 1994; Malhotra et al. 1997; Lahti et al. 2001). In therapeutic trials, agents that selectively enhance NMDA receptor activity have reduced negative symptoms in schizophrenic patients (Cascella et al. 1994; Javitt et al. 1994; Goff et al. 1995; Heresco-Levy et al. 1996; Rosse et al. 1996). The interpretation of these phenomena suggests that abnormalities in NMDA receptor-mediated signal transduction are associated with the pathophysiology of schizophrenia.

PCP induces a psychotomimetic state that closely resembles schizophrenia. Unlike amphetamine-induced psychosis, PCP-induced psychosis appears to reproduce both schizophrenia-like positive and negative symptoms. It therefore has been used as a model to study the neurochemical mechanisms involved in schizophrenia (Javitt and Zukin 1991). PCP was developed in the late 1950s as a general anesthetic that depressed neither respiration nor circulation. However, up to 50% of patients given PCP anesthesia developed severe intraoperative reactions characterized by agitation and hallucinations (Javitt and Zukin 1991). The psychotic state typically lasted 12-96 hours after administration of PCP but occasionally
persisted for 7-10 days (Greifenstein et al. 1958; Meyer et al. 1959). Studies showed that onset of the psychotomimetic effects of PCP often is delayed and that effects last well beyond the peak blood levels of the drug (Meltzer et al. 1972; Wessinger et al. 1991). PCP also has other delayed actions. Olney et al. (1989) reported intracellular vacuolization in the rat posterior cingulate and retrosplenial cortex after administration of PCP. Gao et al. reported its dose-sensitive, delayed action on regional cerebral glucose metabolism of the rat, which occurred over 48 hours, and a late (at 24 hours) change in NMDA and kainate binding in the hippocampal areas (Gao et al. 1993; Gao and Tamminga 1994; Gao and Tamminga 1996). Okuyama et al. (1995) found that PCP produced delayed cognitive dysfunction (at 24 hours) in rats subjected to water maze tasks. These delayed actions of PCP provide information about the mechanism of PCP-induced psychosis, and possibly, about the neurochemical pathophysiology of schizophrenia.

Recently, mRNA differential display has provided a powerful means of identifying and cloning differentially expressed genes (Liang and Pardee 1992). We used this method to identify a gene in rat nucleus accumbens (NAc) that is regulated by the delayed PCP actions. The NAc, the terminal projection site of the mesolimbic dopamine system, is speculated to be critical to the etiology of schizophrenia and critical to reinforcing the properties of drug abuse (Chambers et al. 2001). The NAc has been postulated to be important in schizophrenia because all clinically effective antipsychotics have been known to interfere with dopamine transmission in this region (O’Donnell and Grace, 1998).

Using this approach, we identified synapse-associated protein
90/postsynaptic density-95-associated protein 1 (SAPAP1) (Takeuchi et al. 1997) and found increased SAPAP1 mRNA in rat NAc 24 hours after PCP was administered as well as increased SAPAP1 immunoreactivities in the NAc of patients with schizophrenia. SAPAP/guanylate kinase-associated protein (GKAP) (Kim et al. 1997)/ hDLG- and PSD-95-associated protein (DAP) (Satoh et al. 1997) form a family of proteins that are highly concentrated in the postsynaptic density (PSD) and bind to the guanylate kinase (GK) domain of PSD-95. For simplicity, we here refer to SAPAP/GKAP/DAP as SAPAP. This is the first study to examine the contribution of SAPAP1, one of the NMDA receptor-mediated intracellular signal proteins, to the pathophysiology of schizophrenia.

METHODS

Materials
PCP HCl was a gift from Yamanouchi Pharmaceuticals Ltd. (Osaka, Japan). Haloperidol decanoate was obtained from Dainippon Pharmaceuticals Co., Ltd. (Osaka, Japan). Peroxidase-linked anti-rabbit or mouse Ig (from the donkey) and the enhanced chemiluminescence Western blot detection system were obtained from Amersham (Buckinghamshire, UK), and molecular weight standards from Bio-Rad Laboratories (Richmond, CA). Polyvinylidene difluoride membranes (pore size; 0.45 μm) were purchased from Millipore (Bedford, MA). All other chemicals used were of analytical grade and obtained from commercial sources.

**Animals and treatments**

All experiments were conducted according to the Ethical Guidelines for Animal Experimentation at Kobe University Graduate School of Medicine. Male Sprague-Dawley (SD) rats (300-350 g, CLEA Japan Inc., Tokyo, Japan) were used. The animals were kept in a controlled environment with 12 h dark-light cycles and free access to food and water. For the mRNA differential display and quantitative RT-PCR analyses, rats were acclimated to their surroundings and given a single subcutaneous injection of PCP HCl (10 mg/kg, diluted in sterile saline) or an equal volume of sterile saline. They were decapitated 24 hours later, and their brains were rapidly removed. The NAc, anterior cingulate cortex, hippocampus and caudate-putamen were excised from 1.5 mm-thick coronal cross-sections of the brain with a syringe needle, diameter 1.5mm, as described by Palkovits (1973). Tissues were stored at –80°C until used.

To examine the effects of neuroleptics, rats were given an
intramuscular injection of haloperidol decanoate (30 mg/kg) or the oil vehicle. Twenty-one days after injection, the rats were killed, and their NAc’s excised.

**mRNA differential display**

The RNA image\textsuperscript{R} protocol and kit from GenHunter (Brookline, MA) were used for the mRNA differential display. Isolation of total RNA from rat NAc was done with the TRIzol\textsuperscript{R} Reagent (Gibco, BRL). Total RNA (each 0.5 \( \mu \)g) was converted to cDNA by the use of Maloney murine leukemia virus (MMLV) reverse transcriptase (RT) and an oligo (dT) primer.

One tenth of the cDNA was amplified in a 20 \( \mu \)L total reaction volume containing 1xPCR buffer (10 mM Tris-HCl, 1.5 mM MgCl\textsubscript{2}, pH9.0), 20 pmol of the oligo (dT) primer (T12MN), 4 pmol of an arbitrary hexamer primer, 2 \( \mu \)M dNTP and 1 U Taq DNA polymerase (Amplitaq Gold\textsuperscript{R}; Perkin-Elmer, Norwalk, CT). Cycling parameters were 94\(^\circ\)C for 1 min, 40\(^\circ\)C for 2 min, and 72\(^\circ\)C for 1 min for 40 cycles, then 72\(^\circ\)C for 7 min. The amplified cDNAs were separated in a 6 % DNA sequence gel, after which the gel was stained with SYBR\textsuperscript{R} Green II nucleic acid gel stain (Molecular Probes, Eugene, OR).

Bands representing differentially expressed cDNAs were excised from the gel, reamplified by a PCR with the same primers, and subcloned into the pCR II vector (Invitrogen, San Diego, CA) by TA cloning. Inserts were analyzed by supercoil dideoxynucleotide sequencing.

**cDNA cloning**

A rat brain cDNA library (Uni-ZAP XR Library, Stratagene, La Jolla,
CA) was screened with $^{32}$P-labeled DNA probes from subcloned PCR-derived fragments to isolate full length cDNA clones. Approximately $5 \times 10^5$ clones were screened, and 3 positive clones obtained. DNA was sequenced by the dideoxynucleotide termination method in an Applied Biosystems Prism (ABI PRISM) 377 DNA sequencer (Perkin-Elmer).

**Quantitative RT-PCR**

Quantitative RT-PCR was done with real-time TaqMan technology (Heid et al. 1996), and the analysis was done with an ABI PRISM 7700 (Perkin-Elmer). The method measures PCR product accumulation by use of a dual-labeled fluorogenic probe (the TaqMan probe). The fluorescent reporter FAM (6-carboxyl-fluorescein) located on the 5’ end of the probe is released from a quencher dye (TAMRA, 6-carboxyl-tetramethyl-rhodamine) present on the 3’ end. Total RNA extracted by TRIzol from the rat NAc, anterior cingulate cortex, hippocampus, and caudate-putamen was digested with DNase RQI (Promega). Primers were made from sequences of the isolated clone which corresponded to the 3’-noncoding region of SAPAP1. Sequences of the specific primers were Forward: 5’- CGATAACGCTGTTACCCACTG-3’; Reverse: 5’- TTTGATGAGGGAGACAACTTCTT-3’; and the TaqMan probe: 5’- AAACGTGACAGCGGAACCTTTATTGCGTT-3’. Amplification reactions (50 μL) contained an RNA sample (250 ng each); 5xTaqMan EZ Buffer; 300 μM dATP, dGTP, and dCTP; 600 μM dUTP; 4 mM MgCl$_2$; 5 U rTth DNA polymerase; 0.5 U AmpErase uracil N-glycosylase (UNG); and 200 nM each of the Forward and Reverse primers and 100 nM TaqMan primer. Thermal
cycling was done for 50 cycles of 95°C for 15 s and 60°C for 45 s. As the internal control for the RNA, GAPDH expression was examined under the same conditions. Ratios of SAPAP1 to GAPDH were calculated and normalized.

**Human brains**

Brains were obtained at autopsy from 18 right-handed Japanese patients with schizophrenia (10 men, 8 women, mean age=62.4 years, SD=15.7) and 9 right-handed control subjects (7 men, 2 women, mean age=58.6 years, SD=13.8) who were without any history of neuropsychiatric disorders (Table 1). Consent was obtained from close relatives of the control and schizophrenic patients after the purpose and procedures of our research had been fully explained. The mean postmortem delay was 14.0 hours (SD=9.4) for the patients and 7.8 hours (SD=7.1) for the controls. There was no significant difference between the two groups with respect to age or the time from death to autopsy (Mann-Whitney U test). None of the schizophrenic patients had undergone remission in spite of having been given psychiatric treatment, and before death all had manifested schizophrenic symptoms. The schizophrenia diagnoses corresponded to the DSM-IV category (American Psychiatric Association 1994). Twelve patients had received neuroleptics just prior to death, whereas six had had no medication for at least 3 months before death.

**Antibody**

Rat polyclonal antibody to SAPAP1, raised in rabbits against a GST
fusion of SAPAP1 residues 589-689, was a gift from Dr Y. Takai (Department of Molecular Biology and Biochemistry, Osaka University Medical School, Japan). Antibody against β-actin was purchased by Adcam Ltd. (Cambridge, UK).

**Tissue preparation and Western blot analysis**

At autopsy brains were excised as described elsewhere (Nishino et al. 1986). Briefly, they were removed and stored at −80°C. They next were cut into coronal blocks 1 cm thick on dry ice to obtain tissue blocks. The NAc’s then were excised and stored at −80°C until used. About 1 gram of tissue block was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25°C) containing 1 mM EDTA, 5 mM EGTA, 20 units/ml aprotinin, 20 μg/ml antipain, 20 μg/ml leupeptin, 10 μM N-acetyl-LEU-LEU-norleucinal, 1 mM phenylmethylsulfonyl fluoride, and 25 mM 2-mercaptoethanol. The homogenates were used in the Western blot analysis.

Rat brain samples were homogenized in the same buffer used for the human brain samples. Protein contents of the samples were determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Samples (20 μg protein/lane for SAPAP1; 5 μg protein/lane for β-actin) were fractionated in a sodium dodecyl sulfate/ polyacrylamide gel (SDS-PAGE) (7.5% gel for SAPAP1; 15% gel for β-actin) by the method of Laemmli (1970), transferred to a polyvinylidene difluoride membrane, and the whole membrane was incubated for 90 minutes at room temperature with specific antibodies for SAPAP1 diluted 1:1000. Immunoreactive bands were made visible by an enhanced chemiluminescence Western blot detection
system and scanned with a Scanjet 3c scanner (Hewlett-Packard Co., Greeley, CO). Densitometric readings made with a computer program (NIH Image version 1.59) were confirmed as being within the linear range by processing blots with various concentrations (1-10 μg protein) of the same control sample at the time of the assay. Densitometric readings of the immunoblots on the membranes were normalized by this standard line. Values were expressed as percentages of the mean values for the control subjects. All determinations were done in triplicate.

Statistics

A two-tailed Student’s t test was used to estimate the significance of immunoreactivity difference between the two groups. The significance of differences among the three groups was tested by an analysis of variance (ANOVA) and, if significant, Fisher’s Protected Least Significant Difference (Fisher’s PLSD) was applied.

RESULTS

Differential display and cDNA cloning

mRNA expression 24 hours after the PCP injection was analyzed by differential display PCR. As compared with the controls, three cDNA fragments had greater amplification in rat NAc after PCP injection. One clone, PRP2, was analyzed further (Figure 1). The cDNA fragment was
isolated from a preparative acrylamide/urea gel, subjected to PCR under standard conditions, then subcloned into the pCRII vector via TA cloning. The nucleotide sequence of the clone was AT-rich, which is indicative of a 3’-untranslated region. A sequence homology search with the BLAST program showed it had the highest homology (96.8% for the nucleotide sequences) to human chromosome 18pter-p11.23 clone 34 mRNA (Yoshikawa et al. 1997), a human EST.

To obtain cDNA that had the coding region of the PRP2 gene, a rat brain cDNA library was screened with the radio-labeled PRP2 fragment as the probe, and a cDNA fragment of ~1.4 kb was obtained. A homology search of the data bases showed that this cDNA clone was identical to rat SAPAP1. Therefore, we concluded that it encoded rat SAPAP1.

**Quantitative RT-PCR**

The results of differential display were confirmed by quantitative RT-PCRs done on a large number of rat brains to exclude the possibility that the differentially displayed fragments found represent artifacts sometimes present in differential display applications (Liang and Pardee 1995). Using real-time TaqMan technology (Heid et al. 1996), we estimated the SAPAP1 mRNA in the NAc, anterior cingulated cortex, caudate putamen, and hippocampus, of two matched groups of PCP-treated (n=3) and control (n=3) rats. SAPAP1 expression was measured by the RT-PCR method with an ABI PRISM 7700 (Perkin-Elmer), as described in Materials and Methods. As a reference, GAPDH from the same RNA samples was measured. The expression value calculated for SAPAP1 divided by that for GAPDH was normalized. Results
are expressed as percentages of the values obtained from control rats taken as 100% (Figure 2). Twenty-four hours after the PCP administration, SAPAP1 expression in the NAc and hippocampus were 51% and 13% higher than the expression in the control \( (P<0.0001 \text{ and } P<0.01 \text{, respectively}) \). SAPAP1 expression in the caudate-putamen was increased by 18%, but the difference was not significant. In the anterior cingulate cortex, no such alteration was found. Western blot analysis using a polyclonal antibody to SAPAP1 indicated that, 24 hours after the PCP injection, SAPAP1 immunoreactivity in the rat NAc did not differ significantly from the controls (data not shown).

**Immunooquantification of SAPAP1 in the NAc of patients with schizophrenia using specific SAPAP1 antisera**

Consistent with the results of the differential display method, quantitative RT-PCR analysis showed that SAPAP1 in the NAc was regulated by the delayed action of PCP. SAPAP1 immunoreactivity was measured in brains of patients with schizophrenia to examine whether it is involved in the neurochemical pathogenesis of schizophrenia by Western blot analysis with specific polyclonal antisera against SAPAP1.

Figure 3 shows representative immunoblots with antibody against SAPAP1 in NAcS from control subjects and schizophrenic patients. The most prominent band was at 140 kDa, which is consistent with the size found in rat brain by Takeuchi et al (1997). A faint band of ~100 kDa may represent a different transcript of SAPAP1, DAP1β/GKAP, by alternative splicing. A polyclonal antibody to SAPAP1 was raised against a peptide consisting of amino acids residues 589-689 of the rat SAPAP1 sequence. The amino acid
sequence of residues 589-689 in rat is 89.1% identical to that of human DAP1α/SAPAP1, suggesting that the antigenicity of SAPAP1 in rat and human brains do not differ much.

In the NAcs of patients with schizophrenia, the immunoreactivity for SAPAP1 was 10.1% higher than that of the age-matched controls (Figure 4). The patients with schizophrenia could be divided into two groups, a “medicated” group that had been on neuroleptics at the time of death, and an “unmedicated” group that had not been on neuroleptics for 3 months or more before death. In the unmedicated patients, immunoreactivity of SAPAP1 was 21.0% higher than in the controls ($P=0.010$). In the medicated patients, SAPAP1 did not differ significantly from the value for the controls. There was, however, a significant difference ($P=0.031$) in SAPAP1 immunoreactivity between the 12 medicated and 6 unmedicated patients with schizophrenia. Furthermore, there were no significant changes in the amounts of β-actin between the control subjects and the schizophrenic patients, suggesting that the above-mentioned significant changes indicate a modification of the SAPAP1 immunoreactivity itself. No significant correlation was found between SAPAP1 immunoreactivity and time after death to autopsy ($P>.05$, Spearman’s rank order correlation).

**Effect of neuroleptics on SAPAP1 immunoreactivity in rat brain**

Rats were injected intramuscularly with haloperidol decanoate (30 mg/kg) or an oil vehicle to examine the effect of a neuroleptic. SAPAP1 immunoreactivity in the rat NAc was affected by haloperidol decanoate treatment (after 21 days). SAPAP1 decreased by 18.4% ($P=0.008$), in rats
treated with the neuroleptic as compared with the controls (Figure 5).

DISCUSSION

We used mRNA differential display to identify genes whose transcript levels are regulated by the delayed action of PCP. A candidate cDNA fragment was isolated and sequenced. It carries a 3’-untranslated region and had very close homology to human chromosome 18pter-p11.23 clone 34 mRNA (Yoshikawa et al. 1997). Subsequent screening of a cDNA library showed that it is identical to rat SAPAP1.
SAPAPs (also called GKAP or DAP), a family of proteins identified recently, are expressed in the brain and are highly concentrated in the postsynaptic density (PSD) (Takeuchi et al. 1997; Kim et al. 1997; Satoh et al. 1997). The PSD is a specialized structure beneath the postsynaptic membrane and is crucial to the structural and functional organization of the postsynaptic neurotransmitter receptor proteins and to the adhesion of the postsynapse to presynaptic terminals (Ziff 1997). Studies in various laboratories over the past ten years have identified a number of novel proteins that are part of the PSD. The PSD-95 family of membrane-associated guanylate kinases (MAGUK) have critical roles at the postsynaptic sites of glutamatergic synapses due to their interactions with a variety of ion channels, receptors, cytoskeletal components, and intracellular signaling proteins (Ziff 1997; Sheng and Wyszynski 1997; Craven and Bredt 1998; Kennedy 1998; O’Brien et al 1998). Members of this protein family (PSD-95/SAP90, SAP97/hdlg, PSD-93/chapsyn-110, and SAP102) have a common domain organization of three PDZ domains, a Src homology 3 (SH3) domain, and a GK domain (Scannevin and Huganir 2000). Of these proteins, PSD-95, SAP102, and PSD-93 interact directly with the carboxyl termini (C-termini) of specific NMDA receptor subunits (NMDAR2A and NMDAR2B) both in vitro and in vivo and induce the clustering of NMDA receptors (Kornau et al. 1995; Lau et al. 1996; Niethammer et al. 1996). These findings indicate that MAGUK proteins may be essential for NMDA receptor clustering at synapses (Scannevin and Huganir 2000).

The SAPAP protein family has at least four members and undergoes complex alternative splicing, but the physiological roles of its members are
unknown (Takeuchi et al. 1997; Kim et al. 1997; Satoh et al. 1997). SAPAP interacts with the GK domains of PSD-95 via the middle region that carries 5 repeats of 14 amino acids. SAPAP also binds Shank (Naisbitt et al. 1999)/synamon (Yao et al. 1999) via the C-terminal region. Shank directly interacts with SAPAP and Homer and potentially can bridge the NMDA receptor-PSD95-SAPAP and metabotropic glutamate receptor-Homer complexes in synapses (Naisbitt et al. 1999; Tu et al. 1999). Thus, SAPAPs are thought to be important in the recruiting and clustering of NMDA receptors and MAGUK-based signaling complexes.

In our study, SAPAP1 was drastically increased in the NAc and slightly but significantly increased in the hippocampus due to delayed PCP action. PCP is thought to block the ion channel in the NMDA receptor complex, resulting in diminished glutamatergic neurotransmission at that receptor complex. Increased expression of SAPAP1 mRNA after PCP treatment may reflect up-regulation of SAPAP1 induced by blockade of the NMDA receptor. At a high dose, PCP has not only an effect at the NMDA receptor complex but also other neurochemical effects. The mechanism by which SAPAP1 is increased in the NAc and hippocampus after PCP remains unclear, but changes in the larger neurochemical network in addition to altered glutamatergic transmission may contribute to its mechanism. In contrast, haloperidol decanoate treatment significantly decreased SAPAP1 in the NAc. Antipsychotic drugs could affect glutamatergic neurotransmission by modulating the release of glutamate, by interacting with glutamate receptors, or by altering the density or subunit composition of glutamate receptors (Goff and Coyle 2001). Chronic neuroleptic administration
increases the extracellular glutamate concentration in the NAc (Yamamoto and Cooperman 1994). A recent study found that antipsychotic drugs facilitate NMDA receptor activity by means of an intracellular mechanism (Leveque et al. 2000). Decreased SAPAP1 immunoreactivity in NAcS of rats treated with haloperidol decanoate may reflect an adaptive or compensatory reaction of SAPAP1 to NMDA receptor activation. These findings suggest that SAPAPs may be important in the regulation of glutamatergic neurotransmission via NMDA receptors.

Glutamatergic dysfunction has been suggested to be the basis of the pathophysiology of schizophrenia (Tamminga 1998; Meador-Woodruff and Healy 2000; Goff and Coyle 2001), but it is not clear whether glutamatergic hypofunction results from a primary defect in NMDA receptors or from a defect in one component of NMDA receptor-mediated intracellular signal transduction. Despite the hypothesis that glutamatergic dysfunction in schizophrenia involves the NMDA receptor, relatively few studies have been made of this receptor subtype. Available findings for the NMDA receptor vary markedly, but a consistent feature is that in schizophrenia the obligated NMDAR1 subunit is expressed abnormally in some cortical regions (Meador-Woodruff and Healy 2000). Few studies have been done on the downstream area of the NMDA receptor in brains of schizophrenic patients, but some studies have examined the expression of PSD-95 mRNA in the schizophrenic brain. In situ hybridization analysis showed that PSD-95 expression in the prefrontal cortex was significantly decreased in postmortem material obtained from neuroleptic-treated schizophrenics (Ohnuma et al. 2000). Another study done with quantitative real-time RT-PCR found significantly high mRNA
expression of PSD-95 in the occipital cortex of schizophrenic patients (Dracheva et al. 2001).

In our study, immunoreactivity for SAPAP1 was increased significantly in the NAcs of unmedicated patients with schizophrenia. This suggests that the organizing signaling cascades in the NAcs of patients with schizophrenia may be abnormal and that antipsychotics might reverse this abnormality. Our findings support the hypothesis that there is abnormal glutamatergic neurotransmission in schizophrenia. Moreover, they provide evidence of a potential mechanism by which SAPAPs may govern the pathophysiology of schizophrenia, as well as the neurochemical mechanisms of antipsychotics.

Furthermore, the human SAPAP1 gene is located on chromosome 18p11 which has been linked to both schizophrenia and bipolar disorder (Berrettini et al. 1994; Stine et al. 1995; Schwab et al. 1998; Detera-Wadleigh et al. 1999; Nothen et al. 1999). Berrettini (2000) hypothesized that schizophrenic and bipolar syndromes share certain genetic risk factors and that chromosome 18p11 is one of four genomic regions of overlap for schizophrenic and bipolar susceptibility loci. These findings suggest that SAPAPs are candidate gene sources for schizophrenia.

The study of SAPAP and its relatives promises to increase our understanding of the role of glutamate in the pathophysiology of schizophrenia. Further study is needed for the functional analysis of the human SAPAP gene in patients with schizophrenia.
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FIGURE LEGENDS

Figure 1. PCR differential display analysis of rat brain mRNA 24 hours after a PCP (P) or saline (S) injection. A portion of a typical gel is shown. Differential display was done as described in Materials and Methods. The arrow indicates the high level band present in the PCP-treated sample lane.

Figure 2. Quantitative PCR of SAPAP1 expression 24 hours after a PCP injection as compared to the controls. SAPAP1 expression was measured by the RT-PCR method using the ABI PRISM 7700 (Perkin-Elmer) as described. After examining the amplification plot and standard curve, RNA samples
(50µg) from several tissues after a PCP or saline injection were tested. For references, GAPDH was measured from the same samples. Ratios of SAPAP1 to GAPDH were calculated. The calculated expression level of SAPAP1 after PCP administration divided by that of controls was normalized to 100. Results are expressed as a percentage of the control values taken as 100% and are shown as means ±SD (n=3). Significant differences between the two groups were assessed by means of two-tailed Student’s t test (* \( P<0.0001, \ t=66.91, \ df=4; ** P<0.01, \ t=5.34, \ df=4 \)). NAc: nucleus accumbens; ACC: anterior cingulate cortex; CP: caudate-putamen; Hip: hippocampus.

**Figure 3.** Representative immunoreactive bands for the control subjects and schizophrenic patients determined with antibodies against SAPAP1 and β-actin. Patients were divided into two groups: “medicated”, those who had been on neuroleptics at the time of death and “unmedicated”, those who had not been on neuroleptics for 3 months or more before death. Samples (20µg protein/lane for SAPAP1; 5µg protein/lane for β-actin) of NAcS of the control subjects and schizophrenic patients were fractionated in SDS-PAGE (7.5% gel for SAPAP1; 15% gel for β-actin). Proteins were transferred to a polyvinylidene difluoride membrane, incubated with the specific antibodies, and made visible by an enhanced chemiluminescence Western blot detection system. Molecular sizes (kDa) of marker proteins are given on the left. The immunoreactive bands for SAPAP1 and β-actin were detected at ~140 kDa and ~42 kDa, respectively. Lane 1, control subject; lane 2, medicated patient with schizophrenia; lane 3, unmedicated patient with schizophrenia.
**Figure 4.** Scattergrams of SAPAP1 immunoreactivities in NAcS of the control subjects, and medicated and unmedicated patients with schizophrenia. Results are shown as percentages of the control values. Significant differences in the amounts of SAPAP1 immunoreactivities were observed among the groups by means of ANOVA ($F=4.13$, $df=2$, 24, $P=0.029$), followed by Fisher’s PLSD (*$P<0.05$).

**Figure 5.** Effect of haloperidol decanoate treatment on SAPAP1 immunoreactivities in rat NAcS. SD rats were injected intramuscularly with haloperidol decanoate (30 mg/kg) or an oil vehicle. Twenty-one days after injection, all the rats were killed, and their NAcS excised. Tissue preparation and immunoquantification of SAPAP1 were done as described in Materials and Methods. Results are expressed as percentages of the control values. Significant difference in the amounts of SAPAP1 immunoreactivities were observed between the two groups (two-tailed Student’s $t$ test, $t=2.92$, $df=22$, *$P<0.01$).
Table 1. Autopsy and Clinical Data

<table>
<thead>
<tr>
<th>Gender/Age</th>
<th>Cause of Death</th>
<th>Subtype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Duration of Illness (years)</th>
<th>Medication dose</th>
<th>Duration of Medication (years)</th>
<th>Postmortem Delay (hours)</th>
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<td>59</td>
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\[^a\] M, Male; F, Female.

\[^b\] 295-1, Disorganized type; 295-3, Paranoid type; 295-9, Undifferentiated type.

\[^c\] Off drug. No neuroleptic treatment for at least three months before death.

\[^d\] WND, without neuropsychiatric disease.
Figure 1. Kajimoto Y et al.
Figure 2. Kajimoto Y et al.
Figure 3. Kajimoto Y et al.
Figure 4. Kajimoto Y et al.
Figure 5. Kajimoto Y et al.