

Table 1  
Demographic characteristics of patients with schizophrenia and controls

	Controls	Patients	Statistical value (df)	P value
<i>n</i>	49	27		
Age (years)				
Mean	37.4±12.3	44.3±12.7	<i>t</i> (74)=2.1	0.039
Range	22–72	26–72		
Males/females	14/35	16/11	$\chi^2$ (1)=6.9	0.009
Handedness scale				
Right/left	46/3	26/1		
Educational level (years)				
Mean	16.6±3.4	13.3±2.4	<i>t</i> (73)=4.4	0.00004
Range	12–24	9–18		
Family history +/- <sup>a</sup>		7/20		
Outpatient/inpatient		12/15		
Age of onset (years)				
Mean		23.4±7.7		
Range		13–42		
Antipsychotic medication <sup>b</sup>				
Single dose				
Typical	<i>n</i> =7	782.2±631.4		
Atypical	<i>n</i> =7	421.4±196.1		
Dual dose				
Typical	<i>n</i> =13	529.1±721.0		
Atypical		702.6±606.4		
Number of hospitalizations				
Mean	–	2.9±2.7		
Range	–	0–11		
Hospitalized duration (months)				
Mean	–	82.5±150.8		
Range	–	0–624		

<sup>a</sup> Positive family history was defined as at least one schizophrenic individual within the second degree relatives.

<sup>b</sup> Chlorpromazine equivalent (mg/day).

possible without deviating from the lines. If the subject moved the pencil tip from the space between the lines and could not return it within the lines by himself/herself, the examiner assisted the subject in returning the tip to the lines. In the normal drawing test, the subjects were asked to trace the same hexagon. The subjects were asked to trace the shape as fast as possible without deviating from the lines. In both the mirror and normal drawing tests, the subjects were asked to trace the shape clockwise for the right hand and counterclockwise for the left hand. The time (seconds) to complete the task with his/her dominant hand was recorded as the score for the mirror and normal drawing tests. Although the scores of these motor tests can estimate motor dexterity, they are also dependent on motor speed as mentioned above. Consequently, to examine motor dexterity, we developed the finger movement test, which was derived from a finger movement exercise (Kurita, 1995). The subjects were asked to rotate each finger pair while keeping the remaining fingers fixed (Fig. 2). First, the subjects turned their right and left thumbs together in the same direction five times and then in the reverse

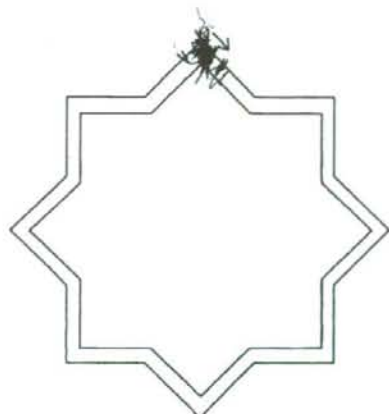


Fig. 1. An example of a drawing test result. This figure is a sample stimulus used in the normal drawing test and the mirror drawing test. The figure was 10 cm in diameter and 3 mm in width. This upper, interrupted drawing was one patient's typical result on mirror drawing. He was unable to complete the drawing and gave up 2 min after starting the test.

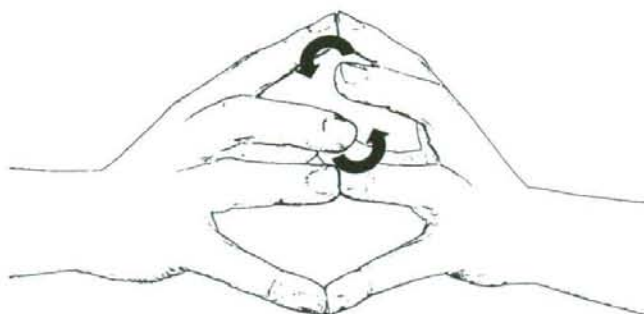


Fig. 2. Finger movement test. The subjects were asked to rotate each finger clockwise and counterclockwise with the remaining fingers extended and fixed. The figure presents the third-finger condition.

direction. The subjects then similarly turned their second, third, fourth, and fifth fingers. An examiner counted the number of separations of the finger pairs that were supposed to remain fixed as a score. The performance for each finger pair was graded as follows: no separation, 5; 1–4 separations, 4–1; 5 or more separations or an inability to do the task, 0. The maximum score was 25 points for individuals who showed no separations. In this test, the time to complete the task was unrelated to the score, making the test result independent of motor speed.

To examine cognitive functions, we administered the Japanese version (Shinagawa et al., 1990) of the Wechsler Adult Intelligence Scale Revised (WAIS-R) (Wechsler, 1981) and the Japanese version (Sugishita, 2001) of the Wechsler Memory Scale Revised (WMS-R) (Wechsler, 1987). To examine executive functions, we administered a computer version (<http://cvddb.shimane-med.ac.jp/user/wisconsin.htm>) of the modified Wisconsin Card Sorting Test (WCST) (Kashima et al., 1987). We analyzed the following measures: the performance IQ, verbal IQ, and full scale IQ from the WAIS-R and the verbal memory, visual memory, general memory, attention and concentration, and delayed recall from the WMS-R, and completed categories from the WCST.

### 2.3. Data analysis

Data were analyzed using SPSS for Windows version 11 (SPSS Japan Inc., Tokyo, Japan). Inter-group differences in means and proportions were assessed with a *t*-test and a chi-square test, respectively. Correlation analysis was performed using Pearson's product-moment correlation coefficient. Analysis of covariance (ANCOVA) was used in comparisons of means to control for confounding variables. To select items that distinguished the patients from the healthy controls, we employed a stepwise discriminant function analysis

using the Wilks' lambda method. The criterion for inclusion/exclusion of *P* value was 0.05. Statistical significance was considered to have been achieved when the two-tailed *P* value was less than 0.05.

### 3. Results

The demographic and clinical data of the patients and controls are shown in Table 1. Mean age, sex ratio, and years of education were significantly different between the two groups.

Table 2  
Comparisons in motor and cognitive tasks between patients with schizophrenia and controls

	Controls	Patients	<i>F</i> <sup>a</sup>	<i>P</i>
<i>A. Motor tasks</i>				
Pegboard	67.0±11.6	132.3±65.6	19.9	3.0×10 <sup>-5</sup>
Normal drawing	15.5±4.0	27.3±9.7	22.9	9.1×10 <sup>-6</sup>
Finger movement	21.7±3.1	13.0±12.9	30.7	5.0×10 <sup>-7</sup>
<i>B. Cognitive tasks</i>				
WMS-R				
Verbal memory	110.2±12.9	76.6±19.5	31.8	3.3×10 <sup>-7</sup>
Visual memory	109.1±11.9	73.4±19.0	42.0	1.1×10 <sup>-8</sup>
General memory	110.8±12.0	72.1±19.2	48.1	1.6×10 <sup>-9</sup>
Attention and concentration	107.3±13.9	83.7±15.0	28.3	1.2×10 <sup>-6</sup>
Delayed recall	112.0±10.1	69.2±18.5	79.0	4.3×10 <sup>-13</sup>
WAIS-R				
Verbal IQ	109.9±13.2	85.0±19.7	23.9	6.1×10 <sup>-6</sup>
Performance IQ	108.2±11.1	76.2±16.8	41.1	1.4×10 <sup>-8</sup>
Full scale IQ	109.9±11.4	79.3±18.9	40.6	1.7×10 <sup>-8</sup>
WCST <sup>b</sup>				
Categories completed	4.1±1.98	2.1±2.17	4.0	0.051

<sup>a</sup> ANCOVA controlling for age, sex, and education years; *df*<sub>1</sub>=1, *df*<sub>2</sub>=70.

<sup>b</sup> ANCOVA controlling for age, sex, and education years; *df*<sub>1</sub>=1, *df*<sub>2</sub>=64.

Table 3

Comparisons in motor and cognitive tasks between patients with schizophrenia and controls matched for age and education level

	Controls	Patients	df	t	P
<b>A. Motor tasks</b>					
Pegboard	67.8±13.2	99.5±31.3	16.1	3.4	<0.01
Normal drawing	16.9±4.2	22.3±7.7	18.7	2.2	<0.05
Finger movement	21.2±3.1	14.8±7.5	16.0	2.8	<0.05
<b>B. Cognitive tasks</b>					
<b>WMS-R</b>					
Verbal memory	107.8±10.0	82.5±21.9	16.9	3.8	<0.01
Visual memory	104.7±15.4	80.6±22.7	24.0	3.2	<0.01
General memory	107.8±11.6	79.9±21.0	24.0	4.2	<0.001
Attention and concentration	106.0±13.6	83.1±14.5	24.0	4.2	<0.001
Delayed recall	110.8±10.4	74.0±20.5	17.8	5.8	<0.0001
<b>WAIS-R</b>					
Verbal IQ	109.2±12.6	90.2±16.8	24.0	3.2	<0.01
Performance IQ	105.9±13.7	81.2±16.2	24.0	4.2	<0.001
Full scale IQ	108.3±12.2	84.9±15.1	24.0	4.3	<0.001
<b>WCST</b>					
Categories completed	3.6±2.3	2.5±2.4	22.0	1.1	0.268

### 3.1. Motor tasks

More than half of the patients (13/23) abandoned the mirror drawing test at an early stage (e.g., Fig. 1), whereas the remaining 10 patients completed the test. By contrast, most of the control subjects (46/49) completed the test, and only three of them failed. This difference was statistically significant ( $\chi^2=15.8$ ,  $df=1$ ,  $P=0.00007$ ). Therefore, although the mirror drawing test could distinguish the patients from the healthy controls, the data of the mirror drawing test were excluded from later analyses. Because significant differences were observed between the patients and control groups concerning age, sex, and educational level (Table 1), the performance in each test was compared between the two groups using an ANCOVA, controlling for these variables. As shown in Table 2A, there were highly significant differences between the two groups in the pegboard, normal drawing, and finger movement tests ( $P=3.0 \times 10^{-5}$ ,  $9.1 \times 10^{-6}$ ,  $5.0 \times 10^{-7}$ , respectively).

### 3.2. Cognitive tasks

Results of the cognitive tests in the total patients and controls are shown in Table 2B. The performance of the patients was significantly poorer than that of the healthy controls in the majority of the cognitive tests, even after controlling for age, sex, and education level in the ANCOVA. Among the cognitive tests, the delayed recall

test was found to be most impaired in the patients. With respect to IQ, deficits in performance IQ were greater than for verbal IQ in the patients. With respect to the WCST, test performance was lower in the patients than in the controls; the difference approached, but did not reach, statistical significance ( $P=0.051$ ).

### 3.3. Pairwise analysis

The observed differences in motor and cognitive tasks between patients and controls may be attributable to the differential distributions in age, sex, and years of education between the two groups. Therefore we reanalyzed the two groups using a pairwise procedure controlling for age, sex and education years. We selected each pair so that differences of age and years of education were within 2 years. Eleven matched pairs were selected. After the pairwise procedure, there was no significant difference between the two groups in age ( $t(20)=0.6$ ,  $P=1.0$ ), years of education ( $t(20)=1.3$ ,  $P=0.2$ ), and sex ratio ( $\chi^2(1)=1.8$ ,  $P=0.2$ ). Table 3 shows the results of  $t$  test analysis of the matched pairs, which gave essentially similar results to the original analysis (Table 2).

### 3.4. Discriminant analysis

We performed a discriminant analysis to select items to distinguish between patients and controls. Two motor tests (finger movement test and pegboard), four memory and intelligence indices (general memory, attention and concentration, delayed recall, and full scale IQ), and one executive test (completed categories of WCST) were put into the stepwise discriminant analysis procedure. In the first step, the delayed recall test was selected. Next, the delayed recall and finger movement tests were selected. These two items correctly classified 96.1%

Table 4

Correlation of demographic and clinical characteristics with delayed recall and finger movement

	Delayed recall		Finger movement	
	r	P	r	P
Age	-0.05	0.81	-0.36	0.06
Educational level (years)	0.51	0.01	0.05	0.80
Age of onset	0.17	0.39	0.03	0.90
Duration of illness	-0.18	0.38	-0.37	0.06
Antipsychotic medication*	-0.33	0.09	-0.04	0.84
Number of hospitalizations	-0.29	0.15	-0.25	0.20
Duration of hospitalization	-0.00	1.00	-0.35	0.07

r = Pearson's product-moment correlation coefficient.

\* Equivalent to chlorpromazine.

Table 5  
Relationship of categorical variables with delayed recall and finger movement

Categories (n)	Delayed recall				Finger movement			
	Mean	S.D.	<i>t</i> <sup>a</sup>	<i>P</i>	Mean	S.D.	<i>t</i> <sup>a</sup>	<i>P</i>
Male (16)	70.3	18.8	0.4	0.719	13.5	6.2	0.3	0.735
Female (11)	67.6	18.8			12.5	8.7		
Outpatient (12)	70.0	17.3	0.2	0.849	17.0	6.9	2.6	0.014
Inpatient (15)	68.6	19.9			9.9	7.0		
Familial history+ (7)	61.6	15.5	1.3	0.209	14.6	6.6	-0.6	0.559
Familial history- (20)	71.9	19.0			12.6	8.1		

<sup>a</sup> *t*-test for independent groups; *df*=2.

of the originally grouped subjects (Wilks'  $\lambda=0.28$ ,  $P=4.9 \times 10^{-19}$ ). When the correlation between the delayed recall and finger movement tests was examined within the patient group, there was no correlation (Pearson's  $r=-0.001$ ,  $P=1.0$ ).

### 3.5. Relationship of demographic and clinical variables with delayed recall and finger movement

The correlations of the demographic and clinical variables (continuous variables) with the delayed recall and finger movement tests are shown in Table 4. While the educational level was significantly correlated with the performance in the delayed recall test ( $r=0.51$ ,  $P=0.01$ ), there was no correlation between years of education and performance on the finger movement test. No significant correlation was observed for the remaining variables.

Differences in performance in the delayed recall and finger movement tests between the groups classified by categorical variables (sex, family history, and outpatient or inpatient status) are shown in Table 5. We found a significant difference in finger movement between outpatients and inpatients (*t*-test,  $P=0.014$ ). The scores of outpatients were significantly higher than those of

inpatients in the finger movement test, although this difference was not observed for the delayed recall test. In regard to sex or family history, there was no significant difference in the scores in either test.

### 3.6. Relationship of motor and cognitive functions with delayed recall and finger movement

The correlations of the motor and cognitive functions with the delayed recall and finger movement tests are shown in Table 6. Finger movement was correlated with pegboard only, while delayed recall was correlated with many of the variables: normal drawing, WMS-R indexes, and full scale IQ.

## 4. Discussion

By using a series of tests, we confirmed that both motor and cognitive functions are profoundly impaired in patients with chronic schizophrenia. The scores of all of the motor (pegboard, mirror drawing, normal drawing, and finger movement) and cognitive (WAIS-R and WMS-R) tests were significantly poorer in the schizophrenic patients than in the healthy control subjects even when sex, age, and years of education were controlled for. A discriminant analysis revealed that the functions that most successfully distinguished patients and controls were delayed recall and finger movement among the tests. Because the finger movement test that we developed is independent of motor speed, our results suggest that motor dexterity is intrinsically impaired in chronic schizophrenia. Furthermore, the score of the finger movement test did not correlate with that of the delayed recall, suggesting that these two functions are dimensionally different.

In accordance with our results, schizophrenia is characterized by a substantial deficit in memory functions (Saykin et al., 1991, 1994; Censits et al., 1997). Among the WMS-R subscales, the most impaired function in our patients was delayed recall (index score of 69.2),

Table 6  
Correlation of motor and cognitive functions with delayed recall and finger movement

	Delayed recall		Finger movement	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Pegboard	-0.27	0.18	-0.51	0.01
Normal drawing	-0.44	0.02	-0.32	0.10
Finger movement	-0.00	1.00	-	-
General memory (WMS-R)	0.88	0.00	0.73	0.72
Attention and concentration (WMS-R)	0.45	0.02	0.20	0.32
Delayed recall (WMS-R)	-	-	-0.00	1.00
Full scale IQ (WAIS-R)	0.65	0.00	0.21	0.30
Completed categories (WCST)	0.34	0.12	0.34	0.11

*r* = Pearson's product-moment correlation coefficient.

followed by visual memory (73.4), verbal memory (76.6), and attention and concentration (83.7). This order is not consistent with previous studies (Wechsler, 1987; Randolph et al., 1994; Hawkins et al., 1997). For example, Gold et al. (1992) studied 45 schizophrenic patients and reported that the scores on the WMS-R subscales scores were similarly impaired (attention and concentration: 80.0; verbal memory: 81.8; visual memory: 81.5; delayed recall: 81.6). The inconsistency between previous studies and the present study may be attributable to the ethnic difference or relatively older age of our subjects (mean age: 44 years). With respect to the WCST, the difference between patients and controls just failed to reach statistical significance ( $P=0.051$ ), although many earlier studies have shown that deficits on the WCST are prominent in schizophrenia (e.g. Franke et al., 1992; Scarone et al., 1993). The failure to reach statistical significance may be attributable to the small sample and relatively large inter-individual differences (i.e., a wide range of standard deviations) in the test scores.

In addition to memory functions, motor functions can be another factor to distinguish schizophrenic patients from healthy controls. The finger movement test we developed was found to have a highly discriminative power between patients and controls; this power might be even higher than that of the conventional motor tests, such as the pegboard test. Motor dysfunction in schizophrenia has been noted since the time of Kraepelin (Kraepelin, 1919; King, 1958; Weaver and Brooks, 1964; Heinrichs and Zakzanis, 1998). Our present results add further evidence for this. The majority of previous studies employed the finger tapping and pegboard tests (Heinrichs and Zakzanis, 1998). Scores on the latter test are, however, influenced by not only dexterity but also motor speed (Rosofsky et al., 1982). Therefore, we developed the finger movement test to evaluate motor dexterity alone. There was a highly significant correlation between the finger movement and pegboard tests, confirming the validity of the newly developed finger movement test as a tool to measure motor dexterity. Our observation that the finger movement test had a highly discriminative power between patients and controls suggests that impairment in motor dexterity *per se* might be a major characteristic of chronic schizophrenia.

However, it could be argued that impaired motor dexterity might have been due to undesirable side effects of the antipsychotic drugs (i.e., extrapyramidal symptoms). Finger rigidity, for example, may influence finger movement test results. Though we found no significant correlation between daily dose of antipsychotic drugs and performance in the finger movement test, there have been conflicting reports concerning the effect of

antipsychotic medication. Some reports showed that motor deficit was independent of antipsychotics (Saykin et al., 1994; Tigges et al., 2000), but others showed that motor deficit in medicated patients was much worse than in drug-naïve patients (Putzhammer et al., 2004, 2005). One plausible explanation was that drug-naïve patients suffer from a primary motor deficit and antipsychotic medication (especially typical antipsychotic medication) worsens this primary deficit (Putzhammer and Klein, 2006). In sum, however, the poor performance observed in the finger movement test is unlikely to be attributed solely to side effects of antipsychotic medication.

With respect to the mirror drawing task, more than half of the patients did not complete it. So we excluded the results on this task from further analysis; however, the observation that so many patients could not complete the task may suggest a major difficulty with visuo-motor coordination in schizophrenia.

There is some evidence that deficits in motor function or dexterity have a developmental origin and manifest before the onset of schizophrenia. Cannon et al. (1999) examined various activities of school age in individuals who later developed schizophrenia, and found that pre-schizophrenic subjects performed significantly worse than control subjects only in nonacademic areas such as sports and handicrafts. Gschwandtner et al. (2005) reported that poorer fine motor functions as well as cognitive functions during childhood were crucial risk factors for later development of schizophrenia. Such deficits in motor functions may be related to genetic factors. Sautter et al. (1997) reported that familial patients were much worse than non-familial patients in the domains of motor and frontal lobe function. In the present study, however, we obtained no evidence supporting this possibility.

In the present study, the inpatients performed more poorly on the finger movement test than the outpatients. In line with this, Weaver and Brooks (1964) reported that scores in motor function tests were highly associated with the probability of discharge from the hospital. Lehoux et al. (2003) studied the relationship of several cognitive and motor tests with social functioning and revealed that the best fitting multivariate model to explain social functioning included fine motor dexterity and executive functioning. Sota and Heinrichs (2004) showed that motor dexterity was one of the crucial cognitive factors which predicted overall quality of life 3 years after initial measurement. These observations may suggest motor dexterity is related to functional outcome.

Several limitations are present in this study. First, the sample size was not very large. There is a possibility that some valid discriminators between patients and controls

have been missed due to the small sample size (type II error). Second, the mean IQ and memory indices of our controls were relatively high (around 110); therefore, it is possible that motor functions might also be relatively higher in our controls than in the general population. Thus, the differences in motor functions between patients and controls may have been overestimated in our study. Third, we did not administer a test for motor speed alone; the possibility remains that motor speed may be an important factor in discriminating patients and controls. Indeed, deficits in motor speed have been reported in both patients with schizophrenia and individuals at risk for the disorder (Niendam et al., 2006). Fourth, scoring of the finger movement test was performed by a single examiner without monitoring by a second examiner or objective instrument; thus some scoring errors might have occurred. Furthermore, strength of the fixed finger might have had some influence on the finger movement test score; controlling for such strength should have been done in the test.

In conclusion, we demonstrated profound impairment in motor dexterity as well as cognitive impairment in chronic schizophrenia using a newly developed motor test, the finger movement test. Impaired motor dexterity is a major characteristic of schizophrenia, which is relatively independent of cognitive functions.

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## p75NTR as a Therapeutic Target for Neuropsychiatric Diseases

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**Abstract:** The p75 neurotrophin receptor (p75NTR) was originally identified as a low-affinity receptor for neurotrophins. Recent studies have revealed that p75NTR can promote cell death or survival and modulate neurite outgrowth depending on the operative ligands and co-receptors. Up-regulation and ligand activation of p75NTR have been shown to be involved in neuronal cell death in cultured cells and animal models of neurodegenerative diseases. The levels of proneurotrophins, which bind to p75NTR to promote neuronal death, have been found to be increased in postmortem brains of patients with Alzheimer's disease. Furthermore, there is some evidence for the involvement of this molecule in psychiatric diseases, such as depression and schizophrenia. Mice lacking *p75NTR* have been shown to have several alterations in central nervous system and cognitive function. Notably, recent progress in genome-based drug discovery has enabled the identification of peptides and non-peptide small molecules targeting p75NTR, which may be potentially beneficial in the treatment of neuropsychiatric diseases. In this review, we focus on recent findings on p75NTR as a therapeutic target for neuropsychiatric diseases.

**Keywords:** p75NTR, neurotrophin, proneurotrophin, depression, schizophrenia, Alzheimer's disease, drug discovery, knockout (KO) mouse.

### INTRODUCTION

p75NTR was identified as a receptor for neurotrophins, namely, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophin 4/5 (NT4/5), and cloned as a type I transmembrane protein, with its molecular weight of 75 kDa being glycosylated through both N- and O-linkages in the extracellular domain [1-5]. Neurotrophins influence numerous cellular activities such as proliferation, growth, differentiation, and regeneration [6]. The most extensively studied neurotrophin, BDNF, for example, has been implicated in Alzheimer's disease [7-9] and psychiatric diseases such as depression [10, 11] and schizophrenia [12, 13]. It is therefore feasible to speculate that the pan-neurotrophin receptor, p75NTR, might play critical roles in the pathogenesis of neuropsychiatric diseases. It might be a possible target molecule for the treatment of such diseases, although little attention has thus far been paid to p75NTR.

### MOLECULAR OUTLINE

Human p75NTR is a 427 amino acid protein containing a 28 amino acid signal peptide, four extracellular cysteine-rich domains (CRD1 to CRD4), an extracellular stalk domain, a single transmembrane domain, and a 155 amino acid cytoplasmic domain. There is a short splicing variant of p75NTR, which will be described in more detail later (Fig. 1). p75NTR binds neurotrophins through interactions with the CRDs, each with six cysteine residues at conserved positions [14, 15]. The extracellular stalk domain is serine/threonine-rich and contains O-linked glycosylation sites [16]. The cytoplasmic juxtamembrane region, called the chopper domain, has been found to be necessary and sufficient to initiate neural cell death [17]. The second half of the intracellular domain is the death domain whose activation induces apoptosis [18, 19]. Signaling mediators, which are activated subsequent to ligand binding to p75NTR, include ceramide [20], nuclear factor  $\kappa$ B (NF- $\kappa$ B) [21], Akt (also known as protein kinase B) [22], c-Jun N-terminal kinase (JNK) [23], and caspases [17].

p75NTR is a receptor for all mature neurotrophins (NGF, BDNF, NT3, and NT4/5) and immature proneurotrophin forms [24] (Fig. 2). Neither tropomyosin-related kinase A (TRKA) nor p75NTR forms a high-affinity binding site when expressed alone, whereas coexpression of the two receptors results in formation of

high-affinity mature neurotrophin binding sites [25]. In forming a complex with TRK receptors (TRKA, TRKB, and TRKC) for the mature neurotrophins, p75NTR modulates the affinity and activity of these kinases that promote neuronal survival [25-30]. The high-affinity binding of proneurotrophins to p75NTR is mediated by interaction of the receptor with a co-receptor, sortilin, which is thought to promote apoptosis [31, 32]. p75NTR can also bind ligands other than neurotrophins, for example, amyloid beta [33], prion peptides (PrPs) [34], and rabies virus glycoprotein (RVG) [35, 36]. Moreover, it interacts with co-receptors other than TRKs and sortilin, for example, the NOGO receptor (NOGOR) and leucine rich repeat and Ig domain containing 1 (LINGO-1) [37, 38]. Further details are described in recent reviews [24, 39].

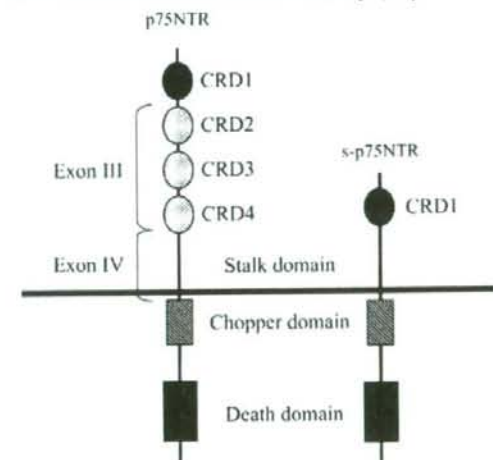


Fig. (1). The structural schema of p75NTR.

Schematic representation of full-length p75NTR and its short splice variant (s-p75NTR)

CRD: cysteine-rich domain

### SPLICING VARIANT

Although p75NTR is mainly expressed as a 75 kDa transmembrane glycoprotein, there is a protein isoform of p75NTR that arises from alternative splicing of exon III (Fig. 1). This isoform is left intact in a *p75NTR* mutant mouse line generated by Lee *et al.* [40,

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41]. In its extracellular domain, the short p75NTR variant (s-p75NTR) differs from the full-length p75NTR protein by the absence of three (CRD2, CRD3, and CRD4) of the four CRDs [42]. Neurotrophins bind to CRD2, CRD3 and CRD4 of p75NTR [15, 43]. Indeed, in HEK293 cells expressing recombinant s-p75NTR, the receptor did not bind to any neurotrophin [40]. Reverse transcription-polymerase chain reaction (RT-PCR) analysis performed on mouse, rat and human cells revealed that s-p75NTR is evolutionarily conserved and coexpressed with full-length p75NTR transcript at different embryonic stages, generally at substantially lower levels. Although the functions of s-p75NTR are largely unknown, some studies suggest that it is a functional molecule *in vivo*. s-p75NTR binds to RVG through CRD1, in contrast to requiring the other three CRDs for neurotrophin binding [36, 44]. Recently, a mammalian homologue of p75NTR, neurotrophin receptor homologue 2 (NRH2), was identified [45-47]. NRH2 contains transmembrane and cytoplasmic domains homologous to those of s-p75NTR; however, it lacks all of the CRDs [45]. NRH2 can interact with p75NTR and Trks, and mediate death or promote survival signals [46, 48].

### KNOCKOUT MICE

Lee *et al.* [41] generated mice lacking functional p75NTR by targeted disruption of exon III, which encodes CRD2, CRD3 and CRD4 (designated henceforth  $p75NTR^{exonIII-/-}$ ).  $p75NTR^{exonIII-/-}$  mice were reported to be viable and fertile, and to develop deficits in heat sensitivity and skin defects in all extremities [41]. Immunohistochemistry revealed a lack of peripheral sensory nerve fibers expressing calcitonin-related peptide alpha (CALCA) and substance P [41]. Neonatal sympathetic and embryonic sensory neurons derived from  $p75NTR^{exonIII-/-}$  mice showed reduced sensitivity to NGF and displayed deficits in developmental and injury-induced apoptosis [49, 50].

Because both the s-p75NTR transcript and its encoded protein are expressed in  $p75NTR^{exonIII-/-}$  mice, von Schack *et al.* [40] targeted exon IV to generate a null mutation (designated henceforth  $p75NTR^{exonIV-/-}$ ). In both  $p75NTR^{exonIII-/-}$  and  $p75NTR^{exonIV-/-}$  mutants, alterations in cholinergic neurons in the basal forebrain, hippocam-

pal neurons, and neurogenic precursor cells in the subventricular zone (SVZ) have been observed (Table I) [40, 41, 49-74].  $p75NTR^{exonIV-/-}$  mice display more severe phenotypes than  $p75NTR^{exonIII-/-}$  mice, particularly in the nervous systems.  $p75NTR^{exonIV-/-}$  mice displayed a larger reduction in the number of dorsal root ganglia (DRG) neurons and Schwann cells, partial perinatal lethality, and defects in the vascular system that have not been observed in  $p75NTR^{exonIII-/-}$  mice [40].

Alzheimer's disease, which causes deficits in learning and memory processes, is accompanied by a loss of cholinergic function [75-77]. Some studies have indicated an increase in the number of cholinergic forebrain neurons in  $p75NTR^{exonIII-/-}$  mice [53, 54, 78], while others reported a decrease [67] or no significant change in the numbers of such neurons [52]. To resolve these conflicting results, Naumann *et al.* analyzed the numbers of cholinergic neurons in the medial septal nucleus of  $p75NTR^{exonIII-/-}$  and  $p75NTR^{exonIV-/-}$  mice on a Sv129/BALB/c genetic background and a back-crossed congenic strain (C57BL/6). The  $p75NTR^{exonIII-/-}$  mutation led to a moderate increase (+13%) in the number of cholinergic neurons only after back-crossing onto a C57BL/6 background. Interestingly, s-p75NTR was present at substantially higher levels in mice with the Sv129 background compared with C57BL/6 mice, which might help to explain this result. In contrast to the  $p75NTR^{exonIII-/-}$  mutation, the  $p75NTR^{exonIV-/-}$  mutation resulted in an over 20% increase in the number of cholinergic neurons, independent of genetic background. They concluded that  $p75NTR^{exonIV-/-}$  mutation increases the number of cholinergic neurons in the medial septum [54].

The  $p75NTR^{exonIV-/-}$  mutation results in severe ataxia in mice and precludes detailed behavioral testing [40]. When spatial learning was examined in  $p75NTR^{exonIII-/-}$  mice, conflicting results were observed, depending on test paradigms. Peterson *et al.*, who found a markedly reduced number of cholinergic septal neurons, reported deficits in acquisition of the Morris water maze, inhibitory avoidance, and habituation tasks in adult  $p75NTR^{exonIII-/-}$  mice [64]. Such deficits in  $p75NTR^{exonIII-/-}$  mice in the Morris water maze were subsequently supported by Wright *et al.* [66]. Performance in the Barnes maze, by contrast, was superior in  $p75NTR^{exonIII-/-}$  mice than in control mice [52]. The Barnes maze is a dry version of the circu-

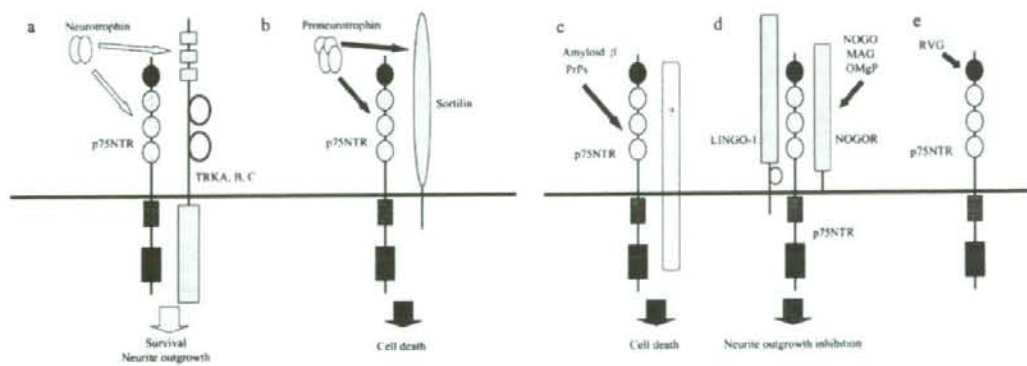


Fig. (2). p75NTR is involved in different biological activities, depending on its different ligands and co-receptors.

- p75NTR physically interacts with TRK receptors (TRKA, TRKB, and TRKC) and enhances their abilities to respond to neurotrophins.
- Interaction of p75NTR with sortilin mediates proapoptotic signals in response to proneurotrophin binding.
- Amyloid beta binds to p75NTR and promotes cell death.
- p75NTR forms a complex with NOGOR that results in growth inhibitory signals to be transduced in response to NOGO, myelin-associated glycoprotein (MAG), or oligodendrocyte myelin-glycoprotein (OMgP).
- RVG binding occurs on CRD1, although this binding is not essential for RV infection.

Table 1. Neuronal and Behavioral Phenotypes of *p75NTR* Knockout Mice

Year	Authors	Region	KO	Phenotypes
1992	Lee, KF et al [41]	SN	III	Marked decrease in sensory innervation by calcitonin gene-related peptide- and substance P-immunoreactive fibers and loss of heat sensitivity
1993	Davies, AM et al [49]	TSN	III	Altered response to NGF in trigeminal sensory neurons
1994	Lee, KF et al [50]	DRG, SCG	III	Excessive loss of DRG and SCG neurons <i>in vivo</i>
1995	Curtis, R. et al [70]	DRG	III	Retrograde axonal transport of NT-4 and BDNF, but not NGF, was dramatically reduced
1997	Yeo, TT et al [53]	BFCN	III	Increase in the number, size, activity, and target innervation of BFCNs
1997	Peterson, DA et al [67]	BFCN	III	Decrease in the number of BFCNs
1997	Bergmann, I. et al [72]	Cutaneous SN	III	Reduction of the epidermal innervation density
1998	Bamji, SX et al [55]	SCG	III	Sympathetic neuron death was developmentally delayed
1998	Fern, CC et al [57]	Facial MN	III	Significant improvement of survival in the adult KO mice, compared to WT, following injury
1999	Brennan, C. et al [65]	SCG	III	In <i>p75NTR</i> <sup>-/-</sup> <i>NGF</i> <sup>-/-</sup> mice, SCG neuron number was restored to WT levels. ( <i>NGF</i> <sup>-/-</sup> mice had 50% fewer SCG neurons.)
1999	Peterson, DA et al [64]	BFCN	III	Impairments in several learning and memory tasks, such as Morris water maze, inhibitory avoidance, motor activity, and habituation tasks
1999	Yamashita, T. et al [71]	SN, MN	III	Reduced outgrowth of sensory and motor axons
1999	Fraide, JM et al [56]	Retina, Spinal cord	III	In <i>p75NTR</i> <sup>-/-</sup> embryos, cell death was reduced in the retina and in the spinal cord
2000	Bentley, CA et al [51]	DRG	III	Abnormalities of axon growth, arborization, and Schwann cell migration during development
2000	Grefenath, U. et al [52]	BFCN	III	Increase in the number of BFCNs, marked increase of the size of BFCNs, and better spatial learning performance
2000	Coulson, EJ et al [69]	DRG	III	Resistance for cell death after withdrawal from cultured medium
2001	von Schack et al [40]	DRG	IV	Larger reduction in the number of DRG neurons and Schwann cells
2002	Naumann, T. et al [54]	BFCN	III, IV	Increase in the number of BFCNs (medial septum), compared with controls
2002	Troy, CM et al [58]	HN	III	Marked reduction in the number of dying neurons after induced seizures
2004	Wright, JW et al [66]	(Behavior)	III	Impairments in Morris water maze task
2005	Zagrebelsky, M. et al [59]	HN	III, IV	Postnatal hippocampal pyramidal cells in both mutant lines had a higher spine density and greater dendritic complexity than WT mice
2005	Rosch, H. et al [60]	HN	III, IV	Hippocampal LTD was impaired in both <i>p75NTR</i> -deficient strains
2005	Woo, NH et al [61]	HN	III	Impairment of the NMDA receptor-dependent LTD and a decrease in the expression of <i>NR2B</i>
2005	Scott, ALM et al [73]	Spinal cord	III	In rhizotomy-treated <i>p75NTR</i> <sup>-/-</sup> mice, intraspinal sprouting was significantly augmented
2006	Sato, T. et al [62]	SGN, HCs	III	Progressive hearing loss, degeneration of SGNs, and severe loss of HCs
2006	Volosin, M. et al [68]	BFCN	III	65% decrease in the number of dying neurons in the medial septum and 80% decrease in the diagonal band compared with the WT mice 1 day after kainic acid treatment
2007	Young, KM et al [63]	NPC in SVZ	III	70% reduction in neurogenic potential <i>in vitro</i> , significant reductions of numbers of PSA-NCAM positive SVZ neuroblasts <i>in vivo</i> , and a lower OB weight
2007	Jansen, P. et al [74]	SCG	III	Significant increase in the number of sympathetic neurons and protection against age-associated cell death

In the KO column, III and IV represent *p75NTR*<sup>non-III</sup> and *p75NTR*<sup>non-IV</sup>, respectively.

BFCN: Basal forebrain cholinergic neuron, CNS: Central nervous system, DRG: Dorsal root ganglia, HC: Ear hair cell, HN: Hippocampal neurons, LTD: Long-term depression, MN: Motor neuron, NMDA: N-methyl-D-aspartate, NPC: Neurogenic precursor cells, NR2B: N-methyl-D-aspartate receptor 2B, OB: Olfactory bulb, PSA-NCAM: Polysialic acid neural cell adhesion molecule, SCG: Superior cervical ganglion, SGN: Spiral ganglion neuron, SN: Sensory neuron, SVZ: Subventricular zone, TSN: Trigeminal sensory neuron, WT: Wild-type.

lar water maze that provides a spatial cue to permit navigation to a safe location. Unlike the study of Peterson *et al.* [64, 67], Grefenath *et al.* [52] did not find a markedly reduced number of cholinergic septal neurons, and reported that cholinergic cells in *p75NTR*<sup>non-III</sup> mice were significantly larger than those in control mice in the medial septal area and in the diagonal band of Broca. They discussed that the improved performance of *p75NTR*<sup>non-III</sup> mice in the Barnes maze correlates with their hypertrophied cholinergic neurons. Taken together, these findings suggest that *p75NTR* may act as a negative regulator of cholinergic neurons in the forebrain.

#### *p75NTR* and Neurodegenerative Disease

Alzheimer's disease is pathologically characterized by extensive neuronal cell death, synaptic loss, intracellular neurofibrillary tangles, and extracellular senile plaques and, as described above, is

associated with a loss of cholinergic neurons resulting in profound memory disturbances and irreversible impairment of cognitive function [75-77, 79-81]. In the postmortem brains of patients with Alzheimer's disease, the levels of proneurotrophins, which bind to *p75NTR* and promote neuronal death, have been found to be increased in several studies [82-86]. In cellular models of neurodegenerative diseases, up-regulation and ligand activation of *p75NTR* have been shown to mediate neuronal cell death [31, 32, 69]. Sortilin, a member of the vacuolar protein sorting 10 protein (Vps10p) domain receptor family, was shown to form a receptor complex with *p75NTR* as an essential component for transmitting proneurotrophin-dependent cell death signals [31, 32]. Sortilin KO (*Sort1*<sup>-/-</sup>) mice were shown to be resistant to age-dependent degeneration of sympathetic neurons [74]. In *p75NTR*<sup>non-III</sup> mice, protection against age-associated cell death was also observed, supporting the possibility of a functional interaction between *p75NTR* and

sortilin in this process [74]. Of note, amyloid beta peptides, the major constituents of senile plaques, have been characterized as ligands for p75NTR [33, 87, 88]. Although this signaling *in vivo* is still unclear, a number of reports have suggested that p75NTR is involved in the promotion of cell death signaling by amyloid beta peptides *in vitro* [89-95]. A recent study provided evidence of a direct link between p75NTR signaling and amyloid beta-induced toxicity in hippocampal neurons *in vitro* and in cholinergic basal forebrain neurons *in vivo* [88]. Both proneurotrophin- and amyloid beta-regulated signaling pathways involving p75NTR *in vivo* seem promising in order to understand the etiology of Alzheimer's disease and to develop novel therapeutic drugs.

#### p75NTR in Neuropsychiatric Diseases

A number of studies indicate that neurotrophins also play an important role in neuropsychiatric diseases such as depression and schizophrenia (reviewed in [7-13, 96]). Therefore, p75NTR, as a pan neurotrophin receptor, might play a key role in neuropsychiatric diseases. As the first study examining a possible association between p75NTR and psychiatric diseases, we reported that a missense polymorphism (S205L) in p75NTR was associated with depressive disorder and attempted suicide in a Japanese population [97]. The frequency of mutant-type (L205) was significantly decreased in patients compared with controls ( $P < 0.05$ , odds ratio 0.54, 95% CI 0.31-0.94), suggesting that this variant may have a protective effect against the development of major depression. Furthermore, this association was more strongly observed in patients with a history of attempted suicide than in those without such a history. A recent study in a North American population, however, failed to obtain evidence for an association between p75NTR polymorphisms, including S205L, and a risk of childhood-onset mood disorder (COMD) or suicide attempt in COMD [98]. In order to clarify the relationship between p75NTR and depressive disorder and suicidal behavior, further studies in large samples are required.

Many lines of evidence indicate that early neurodevelopmental abnormalities contribute to the pathogenesis of schizophrenia [99-101]. Schizophrenia is also characterized by adult-onset subcortical dopaminergic hyperactivity (see, for example, [102, 103]) and disrupted prepulse inhibition (PPI) of acoustic startle (see, for example, [104-107]). p75NTR is widely expressed in the developing central and peripheral nervous systems during the period of synaptogenesis and developmental cell death [108]. Rats treated with neonatal injections of p75NTR antibody conjugated to saporin into the developing prefrontal cortex showed impaired PPI and behavioral changes characteristic of adult-onset dopaminergic hyperresponsivity [109]. It has been suggested that prenatal vitamin D3 depletion can lead to changes in many features of brain development, including morphology, cellular proliferation and neurotrophin systems, which suggests a potential risk-modifying factor for schizophrenia. Interestingly, this change induced by vitamin D3 depletion includes a marked decrease in the expression of p75NTR, and vitamin D3-responsive elements are present in the promoter region of p75NTR [110]. Recently, the early growth response (Egr) transcriptional regulators, Egr1 and Egr3, were identified as direct modulators of p75NTR expression [111]. Egr1 and Egr3 bind to and transactivate the p75NTR promoter *in vitro* and *in vivo* [111]. EGR3 was identified as a potential susceptibility gene in schizophrenia by a recent genetic association study and postmortem brain analysis [112].

Numerous studies have found subregional abnormalities of the brain in patients with schizophrenia, including smaller hippocampal volume, larger ventricles, smaller cerebral volume, reversed asymmetry in the superior temporal gyrus, and smaller volume of the medial temporal lobes (reviewed in [113-115]), and in those with major depression, including reduced volumes of hippocampus, amygdala and anterior cingulate (reviewed in [113, 116, 117]).

There are several lines of evidence suggesting alterations of oligodendrocytes in schizophrenia, for example, lowered density of oligodendroglia [118, 119]. Possibly pertinent to this, proneurotrophins induce death of oligodendrocytes expressing p75NTR [31]. Taken together, these findings suggest that p75NTR might play an important role as a key molecule in such volume changes of the brain in patients with neuropsychiatric diseases.

#### DRUG CANDIDATES

Agonists or antagonists for p75NTR would contain structural determinants of one or more neurotrophin active sites that interact with p75NTR. Longo *et al.* [120] revealed that a peptide corresponding to the region between amino acid residues 28 and 38 of NGF inhibits its neurotrophic effects on DRG neurons. Subsequently, short synthetic peptides corresponding to the beta-hairpin loop of NGF were designed, blocking neuronal death in culture [121]. This NGF-inhibitory activity was p75NTR dependent, requiring both peptide cyclization and dimerization [121]. Turner *et al.* [122] showed that application of a cyclic decapeptide p75NTR antagonist, containing amyloid beta residues 28-30 (Lys-Gly-Ala), protects against NGF-induced death signaling in cultured NSC-34 cells.

Recently, Massa *et al.* [123] identified several small molecules as novel ligands of p75NTR, including a derivative of caffeine, LM11A-24. These compounds are non-peptidyl mimetics of the neurotrophin loop 1 domain identified by tandem *in silico* and *in vitro* screening. LM11A-24 bound to p75NTR, exerted potent neuroprotective effects through one or more p75NTR-dependent mechanisms, and stimulated survival pathways in hippocampal neurons. It also prevented p75NTR-dependent apoptosis induced by proNGF in oligodendrocytes [123]. Subsequently, Pehar *et al.* [124] showed that LM11A-24 was able to inhibit p75NTR-dependent motor neuron death induced by NGF. Intriguingly, the apparent potency of LM11A-24 was considerably higher than that of the above-mentioned peptide-based antagonist containing amyloid beta residues 28-30 (Lys-Gly-Ala) in motor neuron-like NSC-34 cells [123, 122]. LM11A-24 and its related derivatives capable of crossing the blood-brain barrier are expected to become leading candidates in the development of therapeutic strategies targeting p75NTR.

#### CONCLUSION

We focused on recent findings concerning p75NTR in relation to neuronal function, its possible relevance to neuropsychiatric diseases, and progress in genome-based drug discovery targeting p75NTR. p75NTR associates with many kinds of co-receptors and ligands, and transduces various signals, which complicate the understanding of the role of p75NTR *in vivo*. However, in p75NTR KO mice, an increase in the number and size of cholinergic neurons in the medial septum and protection against age-associated neuronal cell death were observed, which raises the possibility that p75NTR might regulate cholinergic neurons negatively in the forebrain. p75NTR, as a mediator of death signaling in both neurons and oligodendrocytes, might contribute to the morphological changes in the brain and subsequent development of neuropsychiatric diseases. Thus, suppression of p75NTR might be a possible therapeutic strategy. Recently, virtual screening *in silico* has been put to practical use in drug discovery and some small molecule ligands for p75NTR have been identified (for example, LM11A-24). Studies on such small ligands for p75NTR with respect to their therapeutic and protective effects in neuropsychiatric diseases are warranted.

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## Impaired Secretion of Brain-Derived Neurotrophic Factor and Neuropsychiatric Diseases

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**Abstract:** Recent studies have elucidated mechanisms of brain-derived neurotrophic factor (BDNF) secretion, and impaired secretion of BDNF may be involved in the pathogenesis of several neuropsychiatric diseases. The huntingtin gene, for example, has been shown to regulate vesicular transport of BDNF, which may play a role in the neurodegeneration present in Huntington's disease. In animal studies, mice lacking calcium-dependent activator protein for secretion 2 (CADPS2), which is involved in the activity-dependent release of BDNF, showed several phenotypes including autistic behavior. A single nucleotide polymorphism that results in an amino-acid change (Val66Met) in the BDNF gene has been shown to cause a decline in the function of BDNF vesicular sorting and has been reported to be associated with behavioral and intermediate phenotypes (e.g., episodic memory) in humans. In this review, we introduce recent progress in the molecular mechanisms of BDNF secretion and discuss its possible role in the pathophysiology and treatment of neuropsychiatric diseases.

### INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, has been implicated in a broad range of processes that are important for neuronal survival and synaptic plasticity in the central nervous system (CNS) [1-3]. Early in the 1950s, nerve growth factor (NGF) was discovered by Levi-Montalcini and Hamburger and Cohen [4,5] as a soluble factor that induced fiber outgrowth of chicken sympathetic neurons. Subsequently, Barde *et al.* [6] isolated BDNF, which was later found to be homologous to NGF [7], from pig brain as a neuronal survival factor. These discoveries motivated homology-based searches for additional family members of which there are currently a total of four in mammals – NGF, BDNF, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). Additional members are conserved in fish – neurotrophin-6 (NT-6) and neurotrophin-7 (NT-7) [8,9]. All neurotrophins are secreted from neuronal (partially glial) cells and bind to their receptors in an autocrine/paracrine manner. In the last two decades, a bulk of studies have suggested that neurotrophins, especially BDNF, are involved in the pathophysiology of neuropsychiatric diseases through their role in the regulation of synaptic efficacy (synaptic plasticity) and synaptogenesis in the CNS. In this review, we focus on recent findings of secretion mechanisms of BDNF and their relationship with neuropsychiatric diseases.

### 1. BIOLOGICAL FUNCTIONS OF BDNF

#### i. Survival and Synaptic Plasticity

Neurotrophins exert their biological effects through the binding of secreted homodimeric neurotrophins to two types

of transmembrane receptor proteins: the tyrosine kinase Trk (tropomyosin-related kinase) receptors and the low affinity common neurotrophin receptor (p75NTR). Neurotrophins are expressed in a precursor form (pro-neurotrophins) and are proteolytically processed to a mature form. Mature neurotrophins preferentially bind to their specific Trk receptor: NGF to TrkA, BDNF and NT-4/5 to TrkB and NT-3 to TrkC. Pro-neurotrophins, however, bind to p75NTR with higher affinity than mature neurotrophins, although they have a lower affinity for Trk receptors [10]. Binding of neurotrophins to Trk receptors immediately generates receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain. Trk receptor phosphorylation activates intracellular signaling regulated by mitogen-activated protein kinase (MAPK), phosphoinositide-3 (PI3)-kinase/Akt and phospholipase C- $\gamma$  (PLC- $\gamma$ ) pathways as well as several small G proteins, including Ras, Rap-1, and the CDC-42-Rac-Rho family [11-13]. These intracellular signaling cascades modulate expression of genes and are responsible for most of the long-term effects of neurotrophins related to neuronal growth, survival, and differentiation [14]. On the other hand, binding of pro-neurotrophins to p75NTR leads to antagonistic effects to Trk receptor signaling. Several of these p75NTR-dependent signaling are pro-apoptotic and can be suppressed by Trk receptor-initiated signaling [15,16]. The first evidence of a significant relationship between neurotrophins and synaptic plasticity was obtained by Lohof *et al.* [17]; exogenous BDNF and NT-3 increased synaptic efficacy at the *Xenopus* neuromuscular junction. Subsequently, these neurotrophins were shown to facilitate glutamatergic synaptic transmission, even in the hippocampus of the mammalian CNS [18-20]. There is now substantial evidence implicating BDNF in activity-dependent long-term synaptic plasticity [21,22]. The neurotrophin-binding Trk receptor activates many kinds of signaling pathways that promote neuronal survival and synaptic efficiency, although it is still unclear how the complex signaling pathways are

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systematically integrated to generate many biological functions.

#### ii. Activity-Dependent Expression of Neurotrophins

Among neurotrophins, BDNF shows the most ubiquitous expression in the developing and adult mammalian brain. BDNF expression levels are increased dramatically during the first few weeks of postnatal development. Expression of neurotrophins in neurons is linked to neuronal activity. BDNF and NGF mRNA levels are rapidly increased by seizure activity in the hippocampus and the cerebral cortex [23-25]. In contrast, blockade of visual input causes rapid down-regulation of BDNF mRNA in the rat visual cortex of dark-reared animals [26]. A similar phenomenon has been found in cultured neurons. The introduction of glutamate with high concentration potassium-induced depolarization increase levels of BDNF and NGF mRNA, while blockade of neuronal activity with  $\gamma$ -aminobutyric acid (GABA) decreases such levels [27,28].

#### iii. Processes of BDNF Secretion

BDNF is synthesized as a 32 kDa precursor protein (proBDNF) and proteolytically cleaved to generate the mature BDNF (13 kDa). The synthesis of the pro-BDNF occurs at the rough endoplasmic reticulum (ER). Following this, pro-BDNF is transported to the Golgi apparatus and concentrated in membrane stacks of the *trans*-Golgi network (TGN). Finally, BDNF-containing vesicles bud off the TGN to eventually transport to the releasing sites. Recent studies clarified some of the details of BDNF vesicular sorting. Specifically, the pro-region of BDNF has been implicated as a regulator of BDNF sorting to secretory vesicles [29]. Moreover, fusing the pro-region of BDNF to NT-4, which is rarely sorted into secretory vesicles, allowed NT-4 to sort more efficiently into specific vesicles [30]. These data support the importance of the BDNF pro-region as a potential target to help guide secretory granules. Furthermore, binding of BDNF to the lipid-raft-associated sorting receptor carboxypeptidase E (CPE) in the TGN is also important for sorting into secretory vesicles of the regulated pathway [31]. Sortilin, a trans-membrane protein, has also been implicated in the sorting of BDNF to secretory granules. Sortilin is expressed in secretory granules and interacts specifically with the pro-region of BDNF. Interestingly, the truncated form of sortilin results in missorting of BDNF to the secretory vesicles [32].

It is still controversial as to where and how pro-neurotrophins are processed into mature neurotrophins in the CNS. Originally, it had been thought that pro-neurotrophins are prototypically cleaved by furin and pro-protein convertases (PCs) in the TGN or in secretory granules before secretion [33]. However, recent studies have indicated that a considerable amount of BDNF is secreted in the pro-form from neurons. Released pro-BDNF is subsequently processed to mature BDNF extracellularly by proteases such as plasmin or matrix metalloproteinases [34,35]. More recently, however, it was shown that pro-BDNF is rapidly converted into mature BDNF intracellularly and almost all BDNF was secreted as the mature form from hippocampal neurons [36].

#### iv. Constitutive and Regulated Secretion

Secretion of neurotrophins is classified into "constitutive" and "regulated" pathways, depending on whether the secretion occurs spontaneously or in response to neuronal activity, respectively. In hippocampal neurons, BDNF appears to be sorted primarily into the regulated pathway [37-39]. In the regulated pathway, BDNF-containing vesicles are transported into either presynaptic axon terminals or postsynaptic dendrites along microtubules for activity-dependent secretion [40-43]. Recently, Lessmann and colleagues conducted an elegant study that provided the long-awaited understanding of BDNF secretion. The activity-dependent postsynaptic secretion of neurotrophins critically depends on  $Ca^{2+}$  influx via ionotropic glutamate receptors or voltage-gated  $Ca^{2+}$  channels,  $Ca^{2+}$  release from internal stores, activation of  $Ca^{2+}$ /calmodulin-dependent protein kinase II (CaMKII), and intact protein kinase A (PKA) signaling. Trk signaling and activation of  $Na^{+}$  channels, on the other hand, are not required for BDNF secretion [44-46]. Furthermore, recent reports suggest that the Golgi apparatus exists in dendrites as well as the cell soma, and have gone so far as to identify a local BDNF secretory pathway in neuronal dendrites [47,48]. Future works may reveal more details concerning the secretory systems of neurotrophins at the subcellular level and that may be more complex and dynamic than we can presently imagine.

## II. IMPAIRED SECRETION OF BDNF AND NEUROPSYCHIATRIC DISEASES

#### i. Huntington's Disease

Huntington's disease (HD) is a fatal, dominantly inherited, neurodegenerative disease that usually presents during midlife. It is characterized by relatively selective degeneration of striatal neurons which lead to psychiatric, cognitive and motor dysfunction. Polyglutamine expansion (polyQ) in the protein huntingtin (htt) is thought to be the principal mechanism for the neuronal toxicity in HD. Recently, evidence has indicated the possible link between HD and BDNF. Wild type htt plays a role as a transcription factor and facilitates expression of BDNF [49]. Furthermore, htt has been implicated in BDNF-containing vesicle transport. Mutant (PolyQ)-htt perturbs post-Golgi trafficking of BDNF in the regulated secretory pathway, though it does not influence the constitutive pathway [50,51]. Conversely, the exogenously transfected BDNF gene generated increased BDNF levels and TrkB signaling in the striatum, which resulted in improved symptoms in HD model mice [52]. These findings suggest that mutation of htt reduces levels of BDNF in the striatum by inhibiting gene expression and perturbing anterograde transport of BDNF-containing vesicles from cortex to striatum. Therefore, the development of therapies focused on the reduction of BDNF release should be important for future studies.

#### ii. Rett Syndrome

Rett syndrome (RTT) is an X-linked disorder characterized by arrested neurological development and subsequent cognitive decline. Methylation of DNA in vertebrates occurs preferentially on cytosine residues of dinucleotides in which the cytosine is followed by a guanine residue (CpGs). Meth-



ylated CpGs bind a variety of proteins. One of these proteins, methyl-CpG binding protein 2 (MeCP2), has been implicated in the long-term silencing of gene expression. Inactivating mutations in MeCP2 is caused in the majority of cases with Rett syndrome. Chen *et al.* showed that MeCP2 selectively binds to the BDNF promoter III and represses expression of BDNF [53]. Membrane depolarization triggers the calcium-dependent phosphorylation and release of MeCP2 from the BDNF promoter III, thereby facilitating transcription [54]. A conditional BDNF transgene increased BDNF expression in the MeCP2 mutant brain, which resulted in rescue of locomotor defects, recovery of electrophysiological deficits, and extension of lifespan in MeCP2 mutant animals [55]. Although MeCP2 null mice exhibited a slightly decreased content of BDNF in some brain areas, mutant neurons demonstrated equivalent secretion levels of BDNF compared to wild-type in response to high-frequency electrical stimulation [56]. Furthermore, BDNF expression in MeCP2 null neurons was significantly improved by chronic ampakine treatment, which was administered to facilitate AMPA receptor activation [57]. These results suggest that the expression of BDNF is still plastic in the MeCP2 null condition and manipulating the BDNF level or the BDNF signaling pathways may provide therapeutic opportunities for RTT patients.

### iii. Autism

Autism is a severe neurodevelopmental disorder with a childhood onset, characterized by profound disturbances in socialization, language skills, communicative, and behavioral functions. BDNF is expressed abnormally in individuals with autism and, as a result, may be involved in the pathogenesis of autism [58,59]. Elevated levels of BDNF and NT4/5 measured by archived neonatal blood samples of autistic patients were reported [60]. Elevation of BDNF was also reported in a study of 18 Japanese children with autism compared with controls [61]. These findings suggest that excess BDNF during childhood may be involved in the neurobiological abnormalities observed in autism. The specific molecular mechanisms involving BDNF and autism remain unknown, though one report suggests that genetic changes in autistic individuals may account for altered neurotrophin levels [62].  $Ca^{2+}$ -dependent activator protein for secretion 2 (CAPS2/CADPS2) is a secretory granule-associated protein that is abundant at the parallel fiber terminals of granule cells in the mouse cerebellum and is involved in the release of BDNF and NT-3. The human CAPS2/CADPS2 gene is located on chromosome 7q31.32 within a critical autism susceptibility locus 1 (AUTS1). CAPS2 knock-out mice demonstrate autistic-like behavioral phenotypes and deficient release of BDNF and NT-3. Moreover, phosphorylation of Trk receptors is decreased in the cerebellum, which may play a role in the pronounced impairment of cerebellar development and function, including neuronal survival, differentiation and migration of postmitotic granule cells, that these mice exhibit [63]. Although there have been few reports suggesting the relation between autism and BDNF secretion, further investigation may result in novel insights.

### iv. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent and unpredictable seizures. Various studies have

shown that BDNF increases neuronal excitability and is up-regulated in areas implicated in epileptogenesis. Seizure activity increases expression of BDNF mRNA and protein, and recent studies have shown that interfering with BDNF signal transduction inhibits the development of the epileptic state *in vivo* [64]. Half of all drug-resistant individuals experience seizure control with dietary manipulation, such as isocaloric substitution of carbohydrates with fats and protein referred to as the 'ketogenic diet'. Daley *et al.* reported that an inhibitor of glycolysis is shown to have antiepileptic effects in the rat kindling model, which may be related to NADH-dependent regulation of BDNF expression [65]. This result may explain how the 'ketogenic diet' treatment works. Although it is unclear whether the up-regulation of BDNF is the cause or the consequence of epilepsy, the reduction of BDNF expression or BDNF signaling can be a useful tool for the treatment of epilepsy.

### v. Psychiatric Disorders

Mood and anxiety disorders are the most common psychiatric diseases. BDNF has been implicated in these disorders, because decreased levels of BDNF in the hippocampus are correlated with stress-induced depressive behaviors [66]. Other studies also showed decreased plasma levels of BDNF in patients with major depression [67]. Many classes of antidepressants, including selective serotonin reuptake inhibitors, significantly increase BDNF mRNA expression in the hippocampus and prefrontal cortex [68,69]. The time course of such increase is consistent with the slow onset of therapeutic effects of antidepressants. More recently, striking evidence for the involvement of TrkB-dependent neurogenesis in the antidepressant effect has been reported. Mice lacking TrkB in the hippocampal neuron progenitor cells had impaired neurogenesis and proliferation induced by antidepressant treatment. These mice also demonstrated increased anxiety-like behavior and decreased sensitivity to antidepressants [70,71]. Taken together, BDNF may play a key role in the brains of recovering patients during antidepressant treatment [72,73].

Many reports have isolated the possible association between BDNF levels and schizophrenia in several brain regions [74]. However, results from these studies are contradictory in that some demonstrate decreased BDNF levels in the postmortem brain or serum, while others report that the BDNF level in patients was not significantly different from that in normal controls [75]. Moreover, samples used in each experiment differ in age, species (rodents, primates, human) and regions (i.e., hippocampus, frontal cortex, CSF and blood) [76]. Although there have been many studies examining the possible role of BDNF in schizophrenia, integrated knowledge concerning this has not been produced. Despite this, the neurobiological vulnerability paradigm remains an attractive concept, supporting that increased susceptibility may be a consequence of reduced expression of BDNF (neurotrophins) at a certain point of life [77].

In the region encoding BDNF's pro-region, a SNP was identified at amino acid 66 (Val66Met). Egan and colleagues reported that the met allele was associated with decreased episodic memory and abnormal hippocampal activation as assayed with fMRI in human subjects [29]. Furthermore, neurons transfected with met-BDNF-GFP showed lower

depolarization-induced secretion, while constitutive secretion was unchanged. Met-BDNF-GFP failed to localize to secretory granules or synapses [29]. Following this, a number of association studies of this polymorphism with psychiatric disorders have been done. Unexpectedly, the Met66 allele, which reduces BDNF release, has been suggested to be protective against developing bipolar disorder [78], although this association was not confirmed by large-scale studies [79,80]. The Met66 allele has also been implicated in other disorders like anorexia [81]. Future research is required to assess how the Val66Met is associated with particular psychiatric disorders.

## CONCLUSIONS

The biological mechanisms of neurotrophins are critically important for neuronal functions that affect brain functions and behavior. Growing evidence has implicated BDNF in the pathophysiology of many neuropsychiatric diseases. Genetic variations leading to deranged expression or secretion due to altered transcription, vesicular sorting, vesicular trafficking and secretion of BDNF seem to play an important role in several neuropsychiatric diseases and related behavioral phenotypes. In order to develop treatment strategies for these diseases through targeting neurotrophins and their receptors, however, clarification of more detailed mechanisms is needed. Studies that reveal not only an increase/decrease in expression of neurotrophins, but also accurate spatiotemporal secretion profiles of neurotrophins are necessary.

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