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ORIGINAL RESEARCH ARTICLE

Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia

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Patients with schizophrenia have reduced neurotrophin levels in their dorsolateral prefrontal cortex (DLPFC) compared to normal unaffected individuals. The tyrosine kinase-containing receptors, trkB and trkC, mediate the growth-promoting effects of neurotrophins and respond to changes in growth factor availability. We hypothesized that trkB and/or trkC expression would be altered in the DLPFC of patients with schizophrenia. We measured mRNA encoding the tyrosine kinase domain (TK+)-containing form of trkB and measured pan trkC mRNA in schizophrenics ($N=14$) and controls ($N=15$) using *in situ* hybridization. TrkB and trkC mRNAs were detected in large and small neurons in multiple cortical layers of the human DLPFC. We found significantly diminished expression of $trkB^{TK+}$ mRNA in large neurons in multiple cortical layers of patients as compared to controls, while small neurons also showed reductions in $trkB^{TK+}$ mRNA that did not reach statistical significance. In normals, strong positive correlations were found between $trkB^{TK+}$ mRNA levels and brain-derived neurotrophic factor (BDNF) mRNA levels among various neurons, while no correlation between BDNF and $trkB^{TK+}$ was found in patients with schizophrenia. TrkC mRNA was also reduced in the DLPFC of schizophrenics in large neurons in layers II, III, V and VI and in small neurons in layer IV. Since neurons in the DLPFC integrate and communicate signals to various cortical and subcortical regions, these reductions in growth factor receptors may compromise the function and plasticity of the DLPFC in schizophrenia.
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We and others have found reduced brain-derived neurotrophic factor (BDNF) levels in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia.[1,2] Chronic changes in neurotrophic factors have been shown to impact the expression of neurotrophic factor receptor genes in the frontal cortex and hippocampus.[3–6] A reduction in cortically derived neurotrophins may have direct consequences for nearby cortical neurons. Therefore, we hypothesized that an altered level of neurotrophin receptor may exist in the DLPFC of patients with schizophrenia. Neurotrophin growth factors bind with high affinity to tyrosine kinase receptors, termed trk receptors (NGF to trkA, BDNF and NT-4/5 to trkB, and NT-3 mainly to trkC) and to a common low-affinity receptor, p75 (for a review, see Chao[7]). TrkB protein exists in two major forms: (1) the catalytic form ($trkB^{TK+}$), which is capable of activating the tyrosine kinase signaling cascade, and (2) a noncatalytic form ($trkB^{TK-}$) that may oppose $trkB^{TK+}$ and block BDNF's ability to activate tyrosine phosphorylation.[8–10] We found abundant $trkB^{TK+}$ mRNA in both large and small neurons, but not in glial cells throughout the prefrontal cortex, whereas the $trkB^{TK-}$ was exclusively localized to astrocyte-like cells.[11] In so far as our classifications may include both pyramidal (large) and nonpyramidal (small) neurons, our observations suggest that both glutamate and GABA-containing neurons in the normal human brain may have the capacity to directly respond to BDNF by activating intracellular tyrosine phosphorylation and associated signaling systems. Indeed trkB mRNA is found in both neuronal types in rodent brain and BDNF can influence the survival, neurite outgrowth and/or synapse formation of both glutamate[12–14] and GABA-containing neurons.[15–18] Since there is evidence for alterations in both excitatory and inhibitory neurons in cortex of subjects with schizophrenia (for reviews, see Blum and Mann[19]; Goff and Coyle[20]), we hypothesized that both large and/or small neurons may be compromised by this BDNF reduction and, in turn, may contain abnormal levels of $trkB^{TK+}$ mRNA in the DLPFC of patients with schizophrenia as compared to controls. To begin to explore how BDNF mRNA levels and

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catalytic trkB receptor mRNA levels may relate, we tested if the levels of these measures correlated.

TrkC is a high-affinity receptor specific for NT-3.[21] While little is known about the developmental or adult expression of NT-3 in the human DLPFC, NT-3 expression is abundant during embryonic and early postnatal brain development in rodents.[22–24] Similar to BDNF, NT-3 stimulates neuritic growth and complexity and induces dendritic growth of cortical neurons in mammals.[25–27] Evidence of abnormal expression of NT-3 protein exists in schizophrenia,[28] and positive genetic associations with the NT-3 gene have been reported in some studies.[29–33] Thus, we also assayed trkC gene expression to address issues of molecular specificity within the neurotrophin receptor family. Indeed, a previously published study has found a 5.8-fold reduction in trkC mRNA by RT-PCR analysis of total RNA extracted from the DLPFC of patients with schizophrenia.[34] We sought to replicate and extend this observation by quantifying trkC mRNA expression by *in situ* hybridization and by determining if any putative change in trkC mRNA was localized to neurons or to any particular cortical layer within the DLPFC of patients with schizophrenia.

Materials and methods

Cohort collection

The cohort for both the $trkB^{TK+}$ and trkC mRNA studies was used in our earlier study of prefrontal BDNF levels in schizophrenia[1] and consisted of 14 patients with schizophrenia and 15 normal controls group-matched for age, sex, race, brain pH and post-mortem interval (PMI, defined as time between death and brain freezing) (Table 1). Post-mortem brains were collected and characterized as either normals or were diagnosed using DSM-IV criteria as previously described.[1,35,36] Out of the 14 patients diagnosed with schizophrenia, three were of the chronic undifferentiated subtype, nine of the chronic disorganized subtype and two of the chronic paranoid subtype. The average age of disease onset was 23 years of age. The total dose of neuroleptic medication given to the patients and brain tissue pH levels were determined as previously described.[35]

Riboprobe design

$trkB^{TK+}$ To avoid cross hybridization to trkA and trkC, we designed a specific $trkB^{TK+}$ (trkB) riboprobe that recognized the tyrosine kinase domain-containing form of trkB.[10,37,38] The cDNA was amplified from human brain total RNA by RT-PCR. The products were inserted into the TA-cloning site of the PCRII vector (Invitrogen, Carlsbad, CA, USA) and were sequenced for confirmation of insert identity. The $trkB^{TK+}$ clone is a 216 base pair template starting at the 3′ end of the transmembrane domain and ending just 5′ to the first tyrosine kinase domain (base pairs 1753–1969, accession #U12140). The forward and reverse primer sequences for amplifying $trkB^{TK+}$ had the following compositions: 5′-CCC-AGC-CTC-CGT-TAT-CAG-C-3′ and 5′-ATG-TTA-TGT-CGC-TTG-ATG-TGC-3′, respectively. The transcript detected by this probe by Northern blot is consistent with the TK+ form of trkB, and has been previously described.[11]

trkC We designed a pan trkC riboprobe that would recognize both the full-length and truncated isoforms of trkC mRNA.[39] The cDNA was amplified from human brainstem total RNA by RT-PCR. The resulting product was inserted into a TA-cloning site of the PCRII vector (Invitrogen, Carlsbad, CA, USA) and was sequenced for confirmation of insert identity. The trkC clone corresponded to a 263 base pair template from the mRNA encoding part of the extracellular portion of the protein (base pairs 110–373, accession #S76476). The forward and reverse primer sequences for amplifying trkC had the following composition: 5′-GCC-CTG-CAA-ATT-GTG-TCT-GC-3′ and 5′GCT-GAA-TGC-TCC-GAA-GTC-CTG-3′, respectively.[39]

Northern blot analysis

A ^{32}P-UTP-labeled antisense riboprobe for trkC was synthesized, with a specific activity of 1.34×10^9 cpm/μg. A multiple tissue blot (Cat# 7755-1, Clonetech, Palo Alto, CA, USA) containing polyA RNA from several brain regions (including frontal, temporal and occipital lobes, putamen, cerebellum, medulla and spinal cord) from the adult human was used not only to verify the existence of the trkC transcript in these regions but also to determine relative regional expression levels. The blot was prehybridized and hybridized as previously described with the addition of trkC riboprobe at 5 ng/ml. The blot was washed stringently as previously described,[35] air-dried and exposed to BioMax film for 2 days. The $trkB^{TK+}$ probe characterization using the same Northern Blot analysis has been published.[11]

In situ hybridization

Six 14 μm-thick tissue sections (two per case per probe) were used for the *in situ* hybridization. Slides were maintained at −80°C until the start date of the procedure and then were thawed, fixed, acetylated, delipidated and dehydrated as previously described by Whitfield *et al*.[40] The ^{35}S-UTP-labeled antisense riboprobes with specific activities of 2.48×10^9 cpm/μg ($trkB^{TK+}$), 2.30×10^9 cpm/μg (trkC), and 1.5×10^9 cpm/μg (cyclophilin, template from Ambion, Austin, TX, USA) were synthesized. In total, 200 μl of hybridization buffer containing the ^{35}S-UTP-labeled riboprobe (5 ng/ml) was pipetted onto each section. Slides were then incubated overnight at 55°C in humidified chambers for hybridization. As a control for specificity of hybridization, we added 5 ng/ml of ^{35}S-UTP-labeled sense riboprobe ($trkB^{TK+}$ and trkC) to additional sections, and treated them the same as the antisense riboprobe. After the *in situ* procedure, slides were exposed to BioMax autoradiographic film (Kodak, Rochester, NY, USA) for 1 week ($trkB^{TK+}$), 1.5 weeks (trkC) and 2 days (cyclophilin), along with ^{14}C

Table 1 Cohort characteristics for human subjects

Brain #	*Diagnosis*	*Age (yrs)*	*Sex*	*Race*	*Side*	*pH*	*PMI (h)*	*Cause of death*	*Manner of death*	*Toxicology*
1	CON	52	F	AA	R	6.87	10.0	Hemopericardium	Natural	Opiates in the occipital lobe
2	CON	35	M	AA	R	5.88	49.5	ASCVD	Natural	Negative
3	CON	41	M	AA	R	6.72	10.0	Stab wounds to chest	Homicide	EtOH
4	CON	42	M	AA	R	6.63	40.0	Acute asthma attack	Natural	Negative
5	CON	66	F	C	R	6.37	29.5	Ruptured thoracic aneurysm	Natural	Negative
6	CON	32	M	AA	R	6.77	15.5	Gunshot wound to chest	Natural	Negative
7	CON	24	M	AA	R	6.59	12.5	Fibrinous pericarditis	Natural	Meperdine in blood
8	CON	38	M	AA	R	6.14	32.5	PE	Accidental	Negative
9	CON	18	M	AA	R	6.51	14.5	GSW to back	Homicide	Negative
10	CON	83	M	AA	L	6.01	66.5	Pulmonary artery thrombosis	Natural	Negative
11	CON	56	M	AA	R	6.09	33.0	PE	Natural	Negative
12	CON	63	M	C	L	6.54	19.0	ASCVD	Natural	Negative
13	CON	52	F	AA	L	6.38	26.0	Acute fibrinous pericarditis	Natural	Lidocaine detected in occipital lobe
14	CON	59	F	AA	R	6.57	37.0	Cirrhosis	Natural	Negative
15	CON	67	F	AA	L	6.69	34.0	Pulmonary edema	Natural	Blood EtOH, 28 mg/dl
Mean		**48.5**				**6.45**	**28.1**			
16	CDS	75	M	AA	L	6.29	41.5	Pending	Natural	Negative
17	CDS	67	F	AA	R	6.63	38.5	Bronchial asthma, COPD	Natural	Negative
18	CDS	31	M	C	R	6.46	14.0	Cerebral edema	Natural	Negative
19	CUS	23	M	AA	L	6.48	42.5	Anoxia due to seizure	Natural	Negative
20	CPS	60	F	AA	L	6.38	19.0	HCVD	Natural	Amantadine detected in brain
21	CUS	30	M	AA	L	6.32	72.5	Pneumonia	Natural	Negative
22	CDS	35	M	AA	L	6.7	79.0	Acute pulmonary embolus	Suicide	Negative
23	CPS	80	M	C	L	6.05	13.5	GI bleed	Natural	NA
24	CDS	81	F	C	R	6.78	11.0	ASCVD	Natural	Amantadine detected in brain, blood negative
25	CUS	61	F	AA	R	6.74	20.0	Asphyxiation, food bolus	Accidental	Negative
26	CDS	38	M	AA	R	6.50	61.0	Ruptured intestine, acute peritonitis	Accidental	Chlorpromazine detected in brain
27	CDS	44	M	AA	R	6.28	37.0	Pulmonary abscess, empyema	Natural	Negative
28	CDS	41	F	AA	R	6.08	51.0	ASCVD	Natural	NA
29	CDS	41	M	AA	L	6.63	32.0	ASCVD	Natural	Negative
Mean		**50.5**				**6.45**	**38.1**			

The group means for age, tissue pH and PMI are given in bold.

standards (American Radiolabeled Chemicals, Inc., St Louis, MO, USA). Autoradiographic films were scanned and analyzed for mRNA expression across cortical lamina according to validated methodology as previously described in detail.[1,11,35] In order to determine trk mRNA levels particularly in neurons, silver grain analysis was conducted on slides as detailed below. Sections for an entire set of cases (29 cases, $n=2$ per case) were processed for *in situ* hybridization at one time.

Silver grain analysis

Slides were dipped in emulsion (NTB-2, Kodak), dried and developed after being in the dark for approximately 6 weeks for $trkB^{TK+}$ or 9 weeks for trkC mRNA. Slides were developed using D-19 developer (Kodak) and Nissl counterstained (with thionin). Analysis of $trkB^{TK+}$ and trkC mRNA expression was accomplished blind to diagnosis using a random starting point within a particular layer of Brodmann's Area 46 (BA46) of the DLPFC. BA46 was determined by the cytoarchitectural criteria described by Rajkowska and Goldman-Rakic.[41] At the microscopic level, we analyzed 10–15 small neurons (layer I–VI) and 30 large neurons (layers II, III, V and VI) resulting in a total of 180–185 neurons per slide (two slides per case were analyzed, thus 10 440–10 730 neurons were surveyed in total for each probe). For this study, large neurons were identified by their large triangular/oblong shape outlined in part by the silver grain label and by light Nissl staining, while small neurons were distinguished by their smaller rounder shape and slightly darker Nissl staining. Identifiable glial cells, which were heavily Nissl-stained, did not have discrete silver grains clustered over them for either probe. If the profile of the cell did not clearly fall into the two neuronal categories as defined above, it was not analyzed. Slides were viewed in bright field at $\times 40$ magnification and silver grains corresponding to the neurotrophin receptor mRNA were counted using a Zeiss Axiophot microscope equipped with a digital camera, a Bioquant Image Analysis System and a personal computer. Large neurons were outlined with a 35 μm diameter circle, and small neurons (defined, in part, as being 15 μm in diameter or less) were outlined with a 15 μm diameter circle. The illumination was switched to dark field and bright pixels above a standard threshold within the delineated circle were counted and converted to number of silver grains by a macroprogram written in Bioquant Image analysis software. Thresholds for silver grain detection were determined by averaging the optimal upper and lower limit from several slides and this threshold was held constant throughout the analysis.

To determine the experimental background level of silver grains, two experimental background measurements were taken by placing either a 35 μm circle (for large neurons) or a 15 μm circle (for small neurons) over an area of neuropil in every layer sampled. The size of the region of interest (ROI) overlying cells was chosen to include a slightly larger area than the diameter of the neuron as radioactivity that is bound to the tissue is known to scatter at all angles and would activate the overlying emulsion such that silver grain deposition would not be constrained by boundaries of the expressing cell. By this sampling method, we included the majority of the silver grains in and around the expressing cell and we had a uniform sampling area with which to compare the background levels of expression. A neuron was considered to be expressing if it contained three times the grains/cell than the averaged experimental background. The percentage of trk mRNA-positive neurons was calculated by the following formula: (# of neurons that reached criteria for expressing/the total number of neurons sampled $\times 100$). Since the majority of neurons reached criteria for expressing neurotrophin receptors (for both trk receptors), we averaged the number of silver grains from all positively expressing neurons in each individual layer for each individual slide, resulting in an average silver grain number for large neurons and small neurons for each layer for each case.

Statistical analysis

The demographics for the normal controls and schizophrenics were compared with independent *t*-tests to verify that the two cohorts were matched for age, PMI and tissue pH. Pearson Product Moment correlations with average $trkB^{TK+}$ and trkC mRNA levels (silver grain/cell) and the demographic variables (age, PMI, pH and freezer time) were run using data from controls only. We ran Spearman's Rank Order correlations (nonparametric) and Pearson Product Moment correlations (parametric) with average $trkB^{TK+}$ and trkC mRNA levels and with estimated lifetime chlorpromazine (CPZ) dose, daily CPZ dose and last CPZ dose. Four separate two-way ANOVAs were performed with diagnosis as the between-groups independent variable, and anatomical layer as the within-group independent variable, and (1) trkC or (2) $trkB^{TK+}$ mRNA levels in large neurons and (3) trkC or (4) $trkB^{TK+}$ mRNA in small neurons as the dependent variables. All significant main effects of diagnosis were followed up by directional Student's *t*-tests to determine in which layer neurotrophin receptor mRNA levels were significantly changed. The one-tailed alpha level was divided by either 4 or 6 according to the number of tests run after each ANOVA for a corrected level of significance threshold of $P=$ or <0.025 for large neurons and a $P=$ or <0.017 for small neurons. Since our groups were group-matched on age, PMI and pH, covariate analysis was not deemed necessary. Finally, separate Pearson Product Moment correlations between BDNF mRNA in each layer and $trkB^{TK+}$ mRNA in each layer (expressed as average grains/cell for each measure) for normal control subjects and for patients with schizophrenia were run.

Results

Northern blotting

Our pan-trkC riboprobe detected one major band in polyA RNA extracted from multiple regions of the adult human brain (see Figure 1a). The size of this band (~4.9 kb) was consistent with the human trkC transcript size previously described in human brain[39] and was found in all regions studied. We also detected a larger, but less abundant, trkC mRNA transcript (>9.5 kb) upon longer exposures to autoradiographic film (see Figure 1b). The adult human cerebellum (lane 1) expressed moderate levels of the ~4.9 kb trkC mRNA while the other subcortical areas (lane 3, 4 and 8) surveyed contained marginally lower levels of this trkC mRNA. The ~4.9 kb trkC mRNA transcript level in the human cerebral cortex was low to moderate and fairly consistent in different lobes, although the frontal (lane 6) and temporal (lane 7) cortex appeared to express slightly more trkC mRNA/μg of poly A RNA than the occipital cortex (lane 5).

Normal anatomy of trkB^{TK+} and trkC expression

TrkB^{TK+} and trkC mRNA were detected in the gray matter of normal human DLPFC, whereas low labeling was found in the layer I and in the subcortical white matter (Figure 2). Sections incubated with radiolabeled sense-strand trkB^{TK+} or with trkC mRNA, used as a control, had no detectable signal (data not shown). For the antisense hybridizations, the trk receptor distribution in layers II–VI was distinct for the two probes (Figure 2, panel a *vs* c, Table 2). As reported previously in normal adults, inspection of the trkB^{TK+} mRNA signal revealed at least one band of increased trkB^{TK+} mRNA signal intensity in the middle cortical layers (arrows, Figure 2a, b and Table 2). In contrast, trkC mRNA appeared to be most densely expressed in layer II, where levels of trkC mRNA from the film analysis were about two-fold above the adjacent layer I (arrows, Figure 2c, d and Table 2). Microscopic observation revealed that silver grains corresponding to mRNAs for full-length trkB and trk C neurotrophin receptors were clearly associated with neurons in all layers including layer 1 as previously reported[11] and silver grains for these two mRNAs were not obviously associated with glia in the adult DLPFC (Figure 3 and as previously reported).[11] Large (where present) and small neurons in layers I, II, III (Figure 3, panels a and b), IV (Figure 3, panels c and d) and V/VI (Figure 3, panels e and f) appeared to abundantly express both trkB^{TK+} and trkC mRNAs. The overall distribution of trkB^{TK+} mRNA (Figure 3, panels a, c and e) and trkC mRNAs (Figure 3, panels b, d and f) appeared fairly similar at the cellular level of resolution. The density of silver grains corresponding to neurotrophin mRNA overlying small neurons, while somewhat variable from cell to cell, was often higher in the small neurons than in the large neurons.

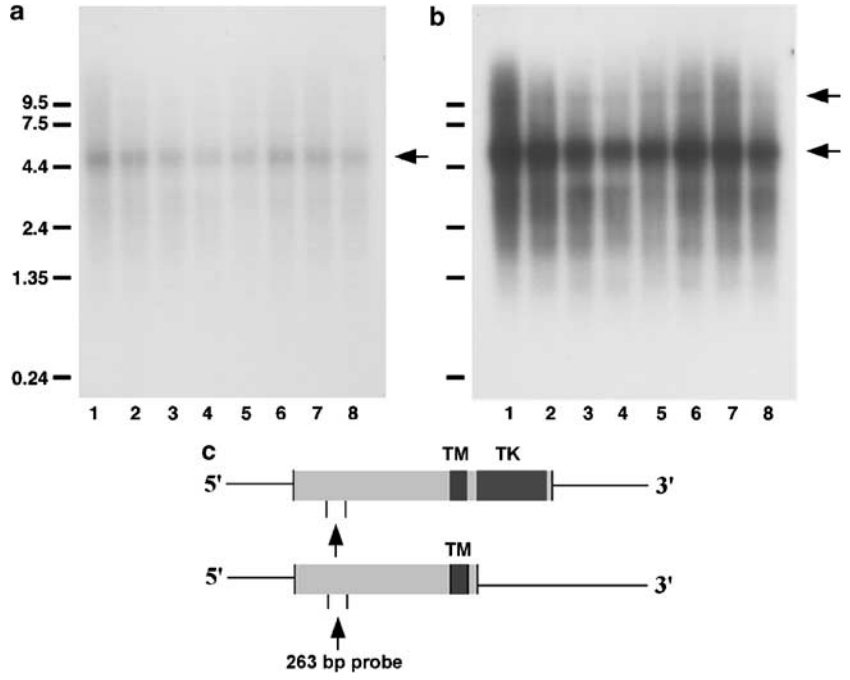
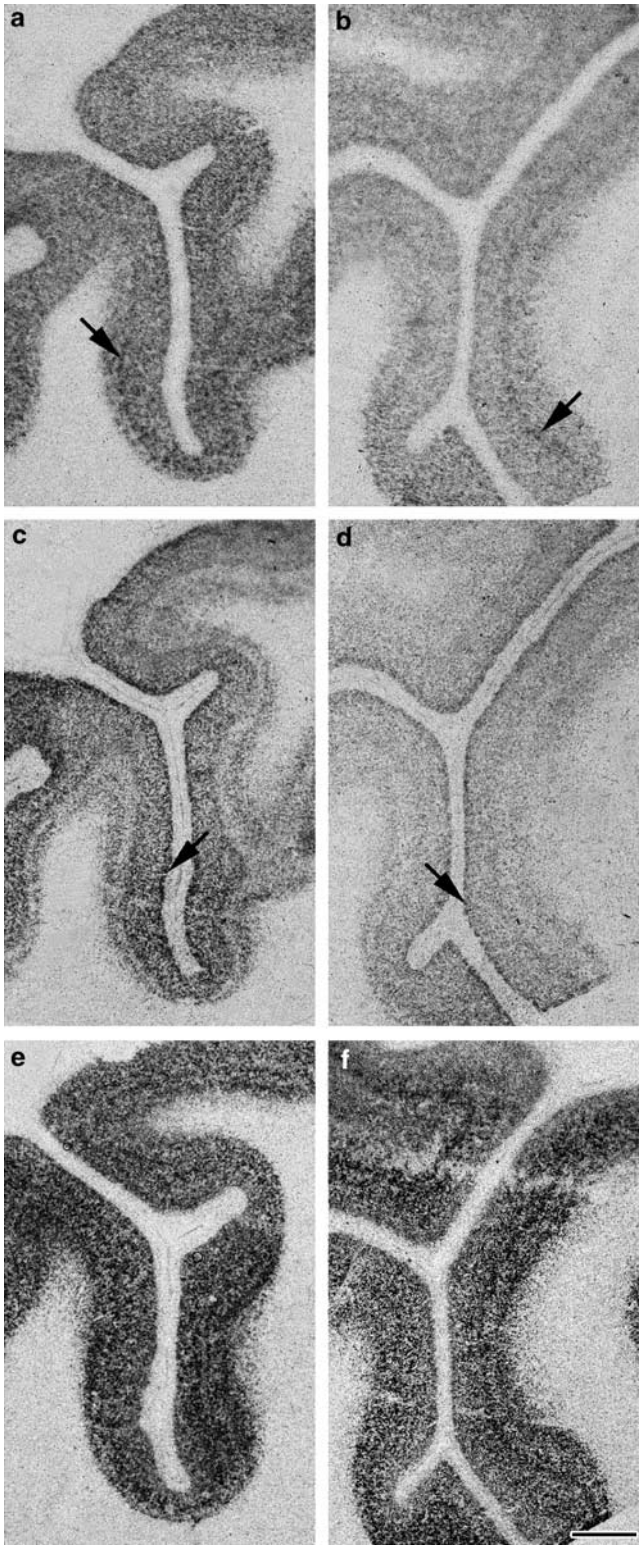


Figure 1 The same Northern blot with our 'pan' trkC riboprobe in multiple brain regions in adult human brain is shown in panel a (shorter exposure) and panel b (longer exposure). At both exposure times, a prominent ~4.9 kb band is found and another less abundant band migrating at ~9.5 kb can be detected at longer exposure times (b). (a, b) Lanes 1–8 contain poly A extracted from cerebellum (1), cerebral cortex (2), medulla (3), cervical spinal cord (4), occipital lobe (5), frontal lobe (6), temporal lobe (7) and putamen (8). (c) The arrow demonstrates approximately where our 263 bp 'pan' trkC riboprobe hybridizes to the trkC transcript (the mRNA encoding the extracellular region of both full-length and truncated protein).

Comparative cyclophilin expression

The quality of the mRNA in tissue slices was evaluated by examining the constancy of cyclophilin expression across all cases. From film-based analysis, cyclophilin mRNA was not found to differ quantitatively in any layer of the DLPFC in patients with schizophrenia as compared to normal controls.[42] The lack of diagnostic difference in cyclophilin mRNA expression in the DLPFC was also found by RNase protection assay performed with subjects from this cohort and with subjects from a similar cohort from our brain collection.[1,35]



Comparative trkB^{TK+} and trkC expression from autoradiographic film

TrkB^{TK+} mRNA expression was reduced in patients with schizophrenia compared to controls in all layers based on analysis of the autoradiographic films; however, this reduction only reached statistical significance in layer VI ($t=2.64$, $P=0.01$). We detected a 13% reduction of trkB^{TK+} mRNA in patients in layer II, a 15% reduction in layer III, a 16% reduction in layer IV, a 21% reduction in layer V (layers I–V, all $P>0.09$) and a significant 29% reduction in deep layer VI. Similar to trkB^{TK+} mRNA, we found that trkC mRNA expression was reduced in patients with schizophrenia across the cortex based on analysis of autoradiographic films. We detected a statistically significant reduction in superficial layer II (24% reduction, $t=2.21$, $P=0.03$) and a trend towards a reduction in layers IV (22% reduction) and VI (21% reduction, both, $P=0.08$). TrkC mRNA expression in layer III (18% reduction, $P=0.13$) and

Table 2 Average laminar values of trkB^{TK+} and trkC mRNA from autoradiographic film

Layer	*Mean trkB^{TK+} mRNA (nCi/g) (SD)*	*Mean trkC mRNA (nCi/g) (SD)*
I	18.5 (11.0)	87.0 (15.8)
II	42.8 (25.3)	194.8 (4.0)
III	52.6 (25.8)	149.2 (15.7)
IV	56.6 (31.5)	131.2 (1.3)
V	52.7 (29.0)	124.5 (9.1)
VI	32.4 (19.9)	108.1 (1.9)

Figure 2 Autoradiographic film images of trkB^{TK+} mRNA (a, b), trkC mRNA (c, d) and cyclophilin mRNA (e, f) are shown, where panels a, c and e are adjacent sections from the same case, as are panels b, d and f. All probes show increased hybridization to cortical gray matter of the middle frontal gyrus as compared to the subjacent white matter in both normals (a, c and e) and schizophrenics (b, d and f). TrkB^{TK+} mRNA expression was seen in normal adult brain in layers II–VI (a) with layer IV having somewhat higher trkB^{TK+} signal (arrow in a and b). The patient with schizophrenia demonstrates a similar but overall less intense pattern of trkB^{TK+} mRNA expression. TrkC mRNA appears most abundant in layer II (arrow in c and d) in normal individuals and in schizophrenics, but is detected at lower levels in the DLPFC of patients. Cyclophilin mRNA is expressed abundantly in layers II–VI in both normals and patients with schizophrenia, and no difference in cyclophilin expression between the two groups was detected. The scale bar in panel f = 1 μm.

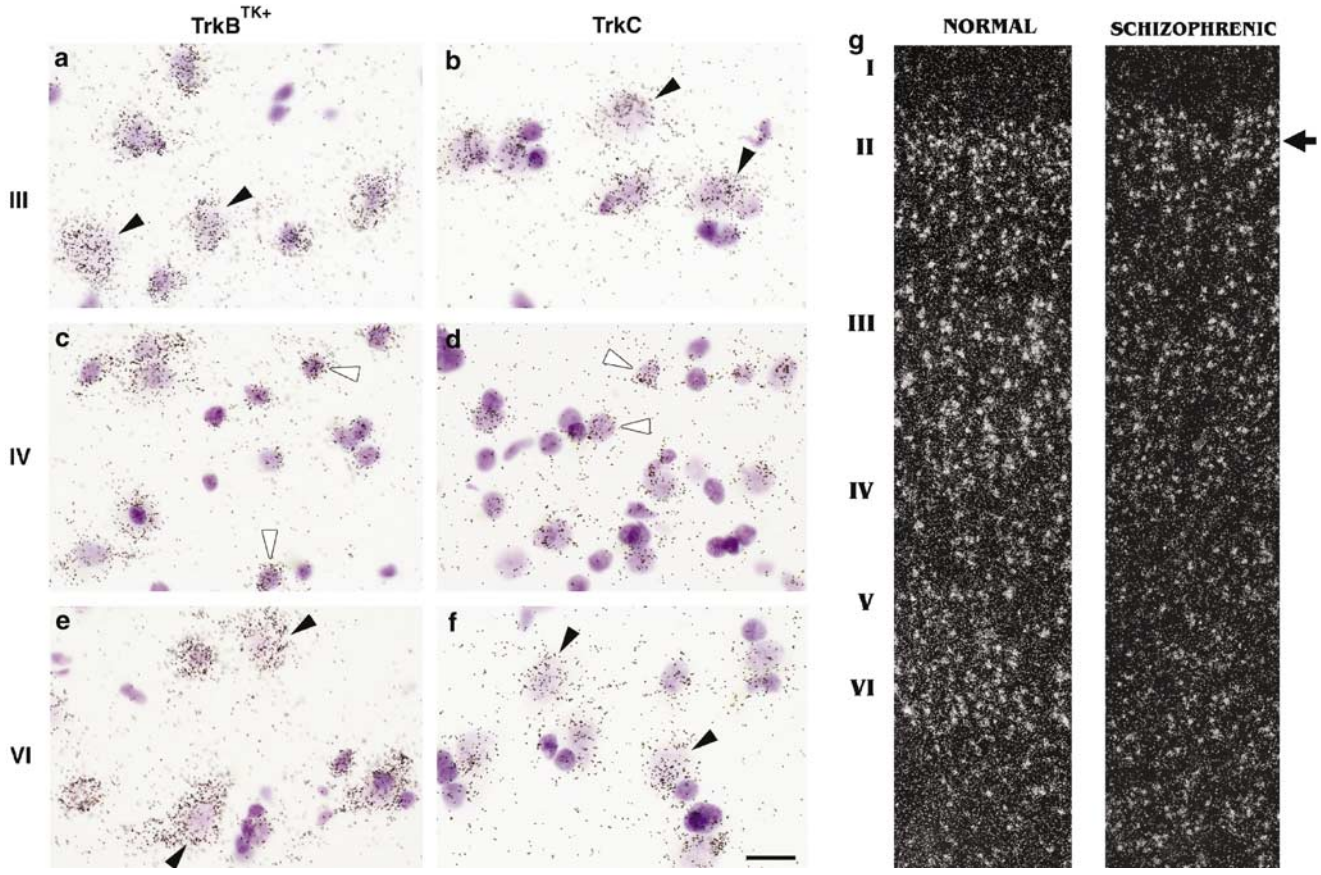


Figure 3 Bright field photomicrographs taken from a Nissl-stained section of a normal adult DLPFC after *in situ* hybridizations ($\times 40$). Silver grains (black dots overlying light purple cells) corresponding to trkB^{TK+} mRNA (panels a, c and e) and trkC mRNA (panels b, d and f) in layers III (a, b), IV (c, d) and VI (e, f) are readily visible. TrkB^{TK+} and trkC mRNA are expressed in large neurons stained lightly with Nissl (black arrowheads) and in small neurons more darkly stained with Nissl (white arrowheads) in multiple layers (scale bar in panel f = 30 μm and applies to panels a–f). (g) Darkfield photomontage of trkC mRNA across cortical layers (I–VI).

layer V (17% reduction, $P = 0.18$) showed a slight reduction in patients with schizophrenia. Since film-based analysis represents mRNA signal coming from different cell types and significant differences in gene expression can be missed when only film-based analysis is relied on (as was the case in our previous study of BDNF mRNA, see Weickert *et al*[1]), we used the more powerful and more cell-specific silver grain quantification method to determine if there was less trkB^{TK+} mRNA and/or trkC mRNA per individual neuron.

Comparative trkB^{TK+} silver grain analysis

Large neurons Patients with schizophrenia had decreased trkB^{TK+} mRNA silver grains in large neurons compared to normal controls ($F = 5.84$, $df = 1, 27$, $P = 0.02$ (Figure 4a)). *Post hoc* Student's *t*-tests analysis revealed a significant reduction in trkB^{TK+} mRNA levels in large neurons in layer II ($t = -3.12$, $df = 1,27$, $P = 0.004$), layer III ($t = -2.40$, $df = 1,27$, $P = 0.024$) and layer VI (($t = -2.44$, $df = 1,27$, $P = 0.022$, (Figure 4a)). The reduction in trkB^{TK+} mRNA in large neurons of patients with schizophrenia varied from 40% in layer II to about 32% in layers III and VI. The comparative analysis for trkB mRNA expression survived Bonferroni correction in layers II, III and VI. Overall, trkB^{TK+} mRNA levels in large neurons varied according to cortical layer ($F = 12.50$, $df = 3,81$, $P < 0.001$). Large neurons in layers III and V had similar levels of trkB^{TK+} grains per cell (Figure 4a), while layer II large neurons had fewer trkB^{TK+} silver grains compared to layers III, V and VI (all $P < 0.002$). There were also a smaller percentage of trkB^{TK+} mRNA-positive large neurons in layer II compared to the other layers (see Table 3). Additionally, layer VI large neurons had significantly less trkB^{TK+} mRNA as compared to large neurons in layer V ($P = 0.01$), and layer VI had a slightly lower percentage of positive cells compared to layer III and V (see Table 3). For trkB^{TK+} mRNA in large neurons, no interaction between cortical layer and diagnosis was detected ($F = 1.87$, $df = 3,81$, $P = 0.14$).

Small neurons In normal and schizophrenic DLPFC, small neurons contained only about 25% of the silver grains detected in the large neurons when expressed

as grains/cell, but had an increased density of label per cell. Overall, the small neurons did not show a significant reduction in trkB^{TK+} mRNA levels in the DLPFC of schizophrenics as compared to normals ((F = 1.56, df = 1,27, $P = 0.22$), (Figure 4c)). However, there was a 17–27% reduction in trkB^{TK+} mRNA levels in the small neurons in layers I, II, III and IV of patients with schizophrenia. For trkB^{TK+} mRNA in small neurons, no interaction between cortical layer and diagnosis was detected (F = 1.24, df = 5,135, $P = 0.29$). We did detect a significant main effect of cortical layer on trkB^{TK+} mRNA levels in small neurons (F = 6.80, df = 5, 135, $P < 0.001$), where layers I and II had significantly less grains per cell as compared to layers III–VI (all $P < 0.05$, except the comparison between layer II and layer V which did not reach statistical significance).

Comparative trkC expression

Large neurons Patients with schizophrenia had decreased trkC mRNA grains/cell in large neurons compared to normal controls (F = 11.04, df = 1,27, $P = 0.003$; (Figure 4b)). In patients, trkC mRNA levels in large neurons were reduced by 22% in layer II ((t = −2.58, df = 1,27, $P = 0.016$), by 25% in layer III (t = −3.42, df = 1,27, $P = 0.002$), by 25% in layer V (t = −4.22, df = 1,27, $P = 0.0003$) and by 21% in layer VI (t = −2.50, df = 1,27, $P = 0.02$, (Figure 4b)). For every layer, the significance of the Student's *t*-test comparisons survived a Bonferroni correction. In the human DLPFC, trkC mRNA levels in large neurons varied across cortical lamina, with neurons in supragranular layers II and III having more grains

Table 3 Percentage of neurons expressing trkB^{TK+} and trkC mRNA (control subjects)

Layer	*trkB^{TK+} Mean (SD)*	*TrkC Mean (SD)*
Large		
II	84.4 (19.8)	89.6 (6.2)
III	92.7 (12.5)	90.8 (8.3)
V	91.4 (11.1)	74.9 (15.6)
VI	88.1 (15.2)	80.2 (12.9)
Small		
I	91.3 (16.1)	58.0 (12.9)
II	93.0 (15.8)	74.3 (13.3)
III	91.7 (15.7)	69.3 (13.7)
IV	94.7 (13.3)	94.0 (5.1)
V	91.3 (16.0)	70.0 (13.7)
VI	91.7 (15.0)	70.3 (21.5)

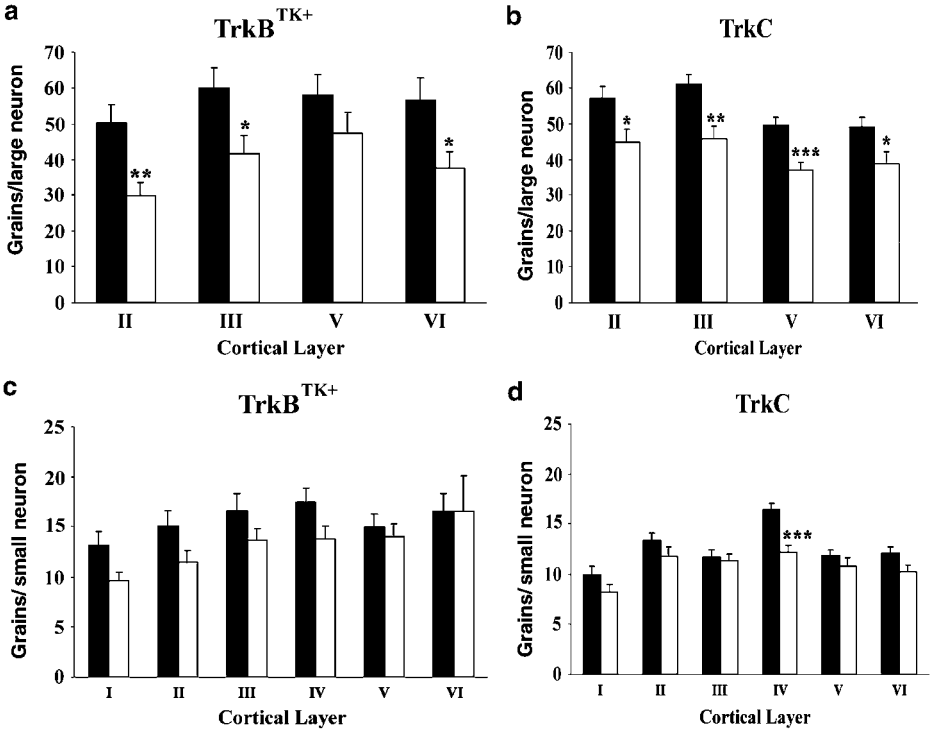


Figure 4 Mean silver grains/cell for large neurons expressing trkB^{TK+} (a) and trkC mRNA (b) and mean grains for small neurons expressing trkB^{TK+} (c) and trkC mRNA (d). Black bars are mean mRNA levels in unaffected individuals and white bars represent mean mRNA levels in patients with schizophrenia. The schizophrenic subjects express less trkB $^{TK+}$ mRNA in large neurons in layers II, III and VI (all $P < 0.025$) (a), less trkC mRNA per large neuron in layers II–VI (all $P \leqslant 0.02$) (b), and less trkC mRNA in small neurons in layer IV ($P < 0.001$) (d). Patients with schizophrenia express levels of trkB^{TK+} mRNA in small neurons that are similar to those in unaffected subjects (c). Error bars represent standard error of the mean. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

per cell than the infragranular layers V and VI (Figure 4b, F = 27.08, df = 3,81, $P<0.0001$). Additionally, the supragranular layers had a higher percentage of trkC mRNA expressing cells than the infragranular layers (Table 3). For trkC mRNA in large neurons, no interaction between cortical layer and diagnosis was detected (F = 1.02, df = 3,81, $P=0.39$).

Small neurons We detected a main effect of diagnosis on trkC mRNA levels in small neurons where patients with schizophrenia had on average a 20% reduction (F = 5.69, df = 1,27, $P=0.024$). We also detected a statistically significant interaction between diagnosis and cortical layer on trkC mRNA levels in small neurons (F = 2.86, df = 5,135, $P<0.017$). *Post hoc* analysis revealed a highly significant 26% reduction in trkC mRNA levels in small neurons that was restricted to layer IV in schizophrenics relative to controls ($t=-4.71$, df = 27, $P<0.0001$ (Figure 4d)). This *post hoc* analysis survived a Bonferroni correction. In both normal and schizophrenic DLPFC, small neurons in layers I, II, III, V and VI contained only about 25% of the trkC silver grains/cell found in large neurons, but similar to $trkB^{TK+}$ mRNA had an increased density of label per cell. The level of trkC mRNA expression significantly varied across the lamina (Figure 4d, F = 19.87, df = 5,135, $P<0.001$). Small neurons residing in layer I expressed significantly less trkC mRNA compared with small neurons in other layers (all $P<0.0002$), while small neurons in layer IV expressed significantly higher levels of trkC mRNA compared to the other small neurons in all other layers ((all $P<0.002$) (Figure 4d)). Furthermore, layer IV had the highest percentage of trkC mRNA-positive small neurons compared to all other layers (Table 3).

Effect of demographic variables

Demographically, the schizophrenic and normal control groups were well matched. The groups did not differ in mean age ($t=0.28$, $P=0.78$), post-mortem interval ($t=1.33$, $P=0.20$) or brain pH ($t=0.008$, $P=0.99$). We tested whether these demographic variables correlated with either $trkB^{TK+}$ or trkC mRNA levels in large neurons, the neuronal population that showed a ubiquitous and robust reduction of neurotrophin mRNAs in schizophrenics compared to normal individuals. Subject age at death and PMI negatively correlated with $trkB^{TK+}$ silver grain levels in large neurons across all layers where $trkB^{TK+}$ mRNA decreased with advancing age (all $r\geq-0.75$, $P\leq0.002$) and with increasing PMI (all $r\geq-0.60$, all $P\leq0.02$). In general, we did not find a significant correlation with $trkB^{TK+}$ mRNA in large neurons and brain pH or freezer storage time, with the exception of freezer time and $trkB^{TK+}$ mRNA layer II ($r=0.52$, $P=0.05$). The pattern of correlations for trkC mRNA with demographic variables was quite similar to $trkB^{TK+}$ mRNA. Age of the subject and PMI negatively correlated with trkC silver grain levels in most layers (II, III and VI, $r\geq-0.52$, $P\leq0.05$, and $r\geq-0.49$, $P\leq0.06$, respectively). We did not detect any significant correlation between trkC mRNA levels in large neurons of layers II, III, V and VI and freezer time or pH.

To determine if the estimated dose of typical neuroleptic drugs related to the expression of $trkB^{TK+}$ or trkC mRNA in both large and small neurons, correlations were run with neuroleptic drug levels (last recorded dose, average daily dose and estimated total lifetime dose expressed in chlorpromazine equivalents). In most layers, $trkB^{TK+}$ and trkC mRNA expression in large neurons did not significantly correlate with last dose, estimated daily dose or estimated lifetime dose of neuroleptics. However, in large neurons and small neurons of layer V, $trkB^{TK+}$ mRNA levels were inversely correlated with total lifetime neuroleptic dose ($r=-0.55$, $P=0.06$ and $r=-0.59$, $P=0.04$, respectively). Since total lifetime dose of neuroleptic is cumulative and therefore higher in older individuals, this correlation may be as much driven by subject age as by drug exposure. To test this, we performed the correlation between neuroleptic dose and $trkB^{TK+}$ mRNA levels while partialing for the effect of age. We found that the correlation in the large neurons in layer V remained about the same ($r=0.60$, $P=0.07$); however, the correlations between neuroleptic dose and $trkB^{TK+}$ mRNA levels in small neurons in layer V in fact showed even stronger correlations when we partialed for the effect of age ($r=-0.69$, $P=0.03$).

TrkC mRNA expression in small neurons of layer III and VI positively and significantly correlated with the estimated total lifetime neuroleptic dose in CPZ equivalents and with the patient's last dose of CPZ ($r=0.75$, $P=0.005$ and $r=0.57$, $P=0.05$, respectively). Since trkC mRNA, like $trkB^{TK+}$ mRNA, decreases with advancing age the positive correlation with lifetime neuroleptic dose and trkC mRNA in patients may represent an effect independent of subject age.

Relationship between BDNF and trkB mRNA

Since BDNF and $trkB^{TK+}$ are trophic partners that can be coregulated, we asked if our previous measurements of BDNF mRNA expressed as grains/cell (made in the same cohort of patients but expressed in large neurons only)[1] correlated with our measurements of $trkB^{TK+}$ mRNA expressed as grains/cell (current study, expressed in both large and small neurons). In normal individuals, $trkB^{TK+}$ mRNA in large neurons of all layers correlated positively with BDNF mRNA levels in layers II, III and V ($r=0.34$–0.59), but not with BDNF mRNA levels in layer VI ($r=-0.05$–0.08). These correlations reached statistical significance between BDNF mRNA in layer II ($r=0.56$) and layer V ($r=0.59$) and $trkB^{TK+}$ mRNA level in large neurons in layer III (see Table 4 for all *r*-values and *P*-values). Significant positive correlations were also detected between $trkB^{TK+}$ mRNA levels in small neurons of layer III and V with BDNF mRNA levels in layer II (Table 4). In dramatic contrast to the overall

Table 4 Correlations between BDNF mRNA and trkB $^{TK+}$ mRNA in cortical layers

Normals

	trkB mRNA in large neurons					
BDNF mRNA	II	III	V	VI		
II	$r=0.45$, $P=0.11$	$\mathbf{r=0.56}$, $\mathbf{P=0.04}$	$r=0.44$, $P=0.11$	$r=0.37$, $P=019$		
III	$r=0.40$, $P=0.16$	$r=0.49$, $P=0.07$	$r=0.38$, $P=0.17$	$r=0.34$ $P=0.24$		
V	$r=0.47$, $P=0.09$	$\mathbf{r=0.59}$, $\mathbf{P=0.03}$	$r=0.45$, $P=0.10$	$r=0.42$, $P=0.14$		
VI	$r=-0.05$, $P=0.86$	$r=0.08$, $P=0.78$	$r=0.06$, $P=0.85$	$r=0.01$, $P=0.97$		
	trkB mRNA in small neurons					
BDNF mRNA	I	II	III	IV	V	VI
II	$r=0.47$, $P=0.09$	$r=0.49$, $P=0.07$	$\mathbf{r=0.54}$, $\mathbf{P=0.05}$	$r=0.33$, $P=0.25$	$\mathbf{r=0.58}$, $\mathbf{P=0.03}$	$r=0.45$, $P=0.11$
III	$r=0.47$, $P=0.09$	$r=0.42$, $P=0.13$	$r=0.45$, $P=0.11$	$r=0.35$, $P=0.23$	$r=0.44$, $P=0.12$	$r=0.29$, $P=0.31$
V	$r=0.51$, $P=0.06$	$r=0.45$, $P=0.11$	$r=0.40$, $P=0.16$	$r=0.41$ $P=0.15$	$r=0.48$, $P=0.08$	$r=0.41$, $P=0.14$
VI	$r=0.04$, $P=0.90$	$r=0.20$, $P=0.50$	$r=0.05$, $P=0.87$	$r=0.03$, $P=0.92$	$r=0.06$, $P=0.85$	$r=0.08$, $P=0.80$

Schizophrenics

	trkB mRNA in large neurons					
BDNF mRNA	II	III	V	VI		
II	$r=0.34$, $P=0.28$	$r=0.31$, $P=0.33$	$r=0.28$ $P=0.37$	$r=0.33$, $P=0.30$		
III	$r=-0.15$, $P=0.65$	$r=-0.26$, $P=0.42$	$r=0.33$, $P=0.29$	$r=-0.36$, $P=0.41$		
V	$r=-0.32$, $P=0.31$	$r=-0.19$, $P=0.56$	$r=-0.20$, $P=0.11$	$r=-0.34$, $P=0.27$		
VI	$r=-$ 0.11, $P=0.74$	$r=0.18$, $P=0.58$	$r=0.15$, $P=0.65$	$r=-0.16$, $P=0.63$		
	trkB mRNA in small neurons					
BDNF mRNA	I	II	III	IV	V	VI
II	$r=0.39$ $P=0.21$	$r=0.22$, $P=0.50$	$r=0.22$, $P=0.49$	$r=0.14$, $P=0.66$	$r=0.23$, $P=0.47$	$r=0.11$, $P=0.74$
III	$r=-0.37$, $P=0.24$	$r=-0.44$, $P=0.15$	$r=-0.21$, $P=0.50$	$r=-0.15$, $P=0.65$	$r=-0.38$, $P=0.23$	$r=-0.15$, $P=0.64$
V	$r=-0.26$, $P=0.41$	$r=-0.48$, $P=0.23$	$r=-0.16$, $P=0.62$	$r=-0.18$, $P=0.59$	$r=-0.32$, $P=0.31$	$r=-0.39$, $P=0.21$
VI	$r=0.04$, $P=0.91$	$r=-0.12$, $P=0.72$	$r=0.06$, P $=0.84$	$r=0.11$, $P=0.74$	$r=0.14$, $P=0.66$	$r=-0.15$, $P=0.87$

The values that reach statistical significance are given in bold.

positive correlations between BDNF and trkB^{TK+} mRNA in normal cortex, the neurons in patients with schizophrenia showed many nonsignificant negative correlations of trkB^{TK+} mRNA levels with BDNF mRNA levels in layers III and V (r range -0.11 to -0.48, Table 4).

Discussion

We report that neurons in the prefrontal cortex of patients with schizophrenia have reduced expression of neurotrophin receptors in multiple cortical layers. In general, we do not find evidence of lamina-specific changes in neurotrophin receptor mRNA from either film-based analysis or from silver grain analysis of large neurons; instead, we find that neurons in all layers show a reduction in both trkB^{TK+} and trkC mRNA. Neurotrophins, acting through trk receptors, can regulate such varied processes as glutamate and GABA neuronal survival, neuronal excitability, somal size, axonal arborization, dendritic architecture, synaptic protein levels and spine density. Morphological and molecular abnormalities of neurons in the DLPFC in schizophrenics include: (1) subtle reductions in somal size,[43,44] (2) reductions in spine density, predominantly in layer III,[45,46] (3) increased neuronal density in the superficial layers II and III[47,48] and (4) reductions in some, but not all, mRNAs and proteins associated with presynaptic terminals.[35,49–51] Our evidence of diminished neurotrophic signaling capabilities in patients with schizophrenia is consistent with these pathological changes.

Both BDNF and trkB knockout mice die early in postnatal life due to peripheral nervous system abnormalities, precluding any study of the final phases of cortical maturation in the homozygous knockout mice. While no major CNS abnormalities have been found in these mice, deficits in the differentiation of interneurons,[52] impaired maturation of Purkinje cell dendrites[53] and an increase in granule cell apoptosis have been reported.[54,55] More recently, forebrain restricted knockout mice for BDNF[56] and trkB[57] have provided evidence that BDNF and trkB are both required for the later stages of cortical development. In the mice lacking BDNF in the forebrain, cortical neurons develop normally until fairly late in postnatal life coincident with the time of normal BDNF induction (about 3–5 postnatal weeks), then the cerebral cortex thins, neuronal density in layers II/III increases, cortical neurons shrink in size and layer II/II large neurons lose basal dendrites.[56] When TrkB is knocked out mainly in large neurons of mice, dramatic thinning of apical dendrites, decreases in dendritic branching, compression of cortical layers II/III and V, smaller neuronal cell size and loss of cortical neurons are found. Many of the cellular changes found when BDNF and/or trkB are 'knocked-out' in the forebrain mimic the cellular changes found in the DLPFC of patients with schizophrenia. Taken together, these results suggest that some of the morphological changes found in the prefrontal cortex of patients with schizophrenia may be downstream of a combined reduction in cortical BDNF and trkB expression.[1]

Reductions in neurotrophin receptor mRNA in patients with schizophrenia could be caused by exposure to neuroleptic drugs, as all of our patients and none of our controls received typical antipsychotic medication. Therefore, this is a potential confound in our study. Clozapine, an atypical antipsychotic, when administered either acutely or chronically did not change trkB or trkC mRNA levels in the cingulate cortex of rodents, but did cause a reduction in trkB mRNA in the olfactory bulb.[13] In our study, the correlations between lifetime neuroleptic exposure and trkB^{TK+} mRNA levels did reach statistical significance. Additionally, significant positive correlations between trkC mRNA in small neurons and neuroleptic exposure estimates were detected; this supports an earlier observation in cerebellum[34], albeit our observation is in cerebral cortex.

Thus, increasing brain trkC mRNA could be part of the therapeutic mechanism of neuroleptic drug action in patients with schizophrenia, but this remains to be tested. The positive correlation between trkC mRNA and antipsychotic drug exposure found by us and others suggests that the neuroleptic drugs are *not* responsible for the decrease in trkC mRNA that we and others find in the brain of schizophrenic subjects.

For other possible confounds, such as PMI, pH and age, all of our cases with schizophrenia did not differ from normals on these variables. A previous study has shown that trkB and trkC mRNAs are stable with postmortem intervals of up to 4 days;[58] thus, PMI may not be a primary determinate of trk mRNA levels in human brain. In support of this, we did not find consistent correlations of PMI and trkB^{TK+} and trkC mRNA across the cortical layers. Similar to our previous analysis of trkB^{TK+} mRNA across the lifespan,[11] we found that age of the subject strongly and negatively correlated with the levels of cortical trkB^{TK+} mRNA. In this study, we find that levels of trkC mRNA in the human DLPFC may be similarly influenced by aging. Thus, both trkB^{TK+} and trkC mRNA appear to decrease somewhat linearly during the third to ninth decade of life. The significance of this neurotrophin receptor reduction to the aging process in the human cortex is not known.

One important question raised by recent studies on neuronal growth factors in schizophrenia is: How do the changes in neurotrophins and neurotrophin receptors relate to each other, if at all? Studies in rodent brain have shown that changes in BDNF mRNA and trkB mRNA occur in parallel;[4,5] however, trkB mRNA can also be downregulated when BDNF is increased.[3,5] We hypothesized that the reduction in the levels of BDNF mRNA in the schizophrenic cortex may impact receptor synthesis in nearby neurons. Indeed, we find consistent positive correlations between BDNF and trkB^{TK+} mRNA levels in normal individuals, suggesting that their expression may be linked in the frontal cortex. For example, cortical

neurons in both superficial and deep layers, which can be directly interconnected,[59] show positive correlations between BDNF mRNA levels and trkB^{TK+} mRNA, suggesting that trophic relationships both within superficial and spanning superficial and deep lamina may exist. Contrary to our prediction that the reduction of BDNF and trkB^{TK+} mRNA in the DLPFC of patients with schizophrenia would be positively correlated, these two measures are not correlated in the disease state and even show a tendency towards a negative relationship. This suggests that there may be a collapse of normal coordinated BDNF–trkB^{TK+} signaling and gene regulation in the DLPFC of the schizophrenic brain. We stress that these correlations (or lack thereof) should be viewed as preliminary in nature as correlations do not prove causality and many correlations were run overall.

BDNF is trophic for GABA-containing interneurons,[15–18,60] and these neurons synapse directly on nearby large neurons that synthesize BDNF. Fairly consistent evidence exists for pathology in GABA-containing interneurons in the brains of patients with schizophrenia,[61–69] and the reduction in BDNF may have consequences for this population of neurons. We find a similar magnitude of trkB^{TK+} mRNA reduction in small neurons in the superficial layers I–IV as we do in the larger neurons. Indeed, a recently published study showed a similar reduction in trkB mRNA in the DLPFC of patients with schizophrenia as we report here and, in addition, these authors showed that the trkB mRNA levels were strongly correlated with GAD-67 mRNA levels.[2] Neither our study nor the previously published study directly addressed if GABA-containing neurons contain less trkB^{TK+} mRNA in patients with schizophrenia as this would require double-labeling. Although we do find that small neurons express trkB^{TK+} mRNA, we do not know what proportion of these were in fact GABA-containing inhibitory neurons. Testing whether neurotrophin receptor mRNA levels are altered in definitive GABA-containing interneurons in schizophrenia is an important question for future research. However, even this approach may have limitations as various subpopulations of interneurons are known to normally express trkB and trkC mRNA to varying extents,[70] and averaging expression levels across various subpopulations of interneurons may result in increased variability and reduced power to find a change in inhibitory neurons overall.

Another group has previously reported significantly reduced levels of trkC mRNA in the frontal cortex of schizophrenics.[34] We have replicated and extended their initial observation to show that large neurons in all layers and small neurons in layer IV contribute to the trkC mRNA reduction. We did not detect the same magnitude of reduction using *in situ* hybridization as Schramm and co-workers detected using RT-PCR ($\sim$5.8-fold *vs* $\sim$23% in our study). This difference may reflect the fact that the RT-PCR is carried out in homogenates and could include the trkC mRNA expressed by glial cells, whereas in our quantitative study on tissue slices, trkC mRNA signal in glial cells was not significantly above background and we focused exclusively on neuronal trkC mRNA expression. Since large neurons surveyed in our study may represent primarily pyramidal neurons, we suggest that excitatory neurons contain less trkC mRNA in the prefrontal cortex of patients with schizophrenia compared to controls. However, a recent study of trkC mRNA from autoradiographic film-based analysis has failed to find a significant reduction in trkC mRNA in the DLPFC of patients with schizophrenia,[2] suggesting that not all cohorts may demonstrate a reduction in trkC mRNA or that methodological differences may impact the ability to detect trkC mRNA changes in the brains of patients with schizophrenia.

Our study suggests that 84–93% of all large neurons in the human DLPFC express trkB^{TK+} receptor mRNA, and that 75–91% of this same population express trkC receptor mRNA. Hence, most large neurons would be predicted to be responsive to both TrkB ligands (BDNF and NT4/5) and also, to TrkC ligands (NT-3). Indeed, the same cortical pyramidal neuron can express both trkB and trkC receptors;[71] however, the significance of this is not known. Some studies suggest that BDNF and NT-3 can have opposing roles in cortical neuron growth,[18,27,72] while other studies suggest that they cooperate in the elaboration and branching of cortical neurons.[73] The major ligand for trkC, NT-3, was reported to be significantly lower in patients with schizophrenia as compared to controls in frontal and parietal cortex.[28] Interestingly, a link between the NT-3 gene and schizophrenia has been reported in most,[29–33] but not all association studies.[74] However, it is not known if variation in the NT-3 gene is a primary component of the disease process in schizophrenia, nor is it known how any putative change in NT-3 may relate to changes in NT-3 protein levels reported by others or to the reduction in trkC mRNA that we report here.

In summary, we find widespread reduction in neurotrophic factor receptor gene expression in neurons of the DLPFC in patients with schizophrenia. Reductions in neurotrophin receptor synthesis may in turn lead to reduced ability of frontal cortical neurons to respond to neuronal growth factors in this disorder. These putative alterations in neurotrophin signaling ability could result in impaired synaptic communication and impaired cellular function. Thus, this fairly ubiquitous reduction in neurotrophin receptors within the DLPFC may relate to the morphological, physiological and functional disruption known to exist in the prefrontal cortex in schizophrenia.

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