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Reduced GAP-43 mRNA in Dorsolateral Prefrontal Cortex of Patients with Schizophrenia

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Schizophrenia has been associated with anatomical and functional abnormalities of the dorsolateral prefrontal cortex (DLPFC), which may reflect abnormal connections of DLPFC neurons. We measured mRNA levels of growth-associated protein (GAP-43), a peptide linked to the modifiability of neuronal connections, in post-mortem brain tissue from two cohorts of patients with schizophrenia and controls. Using the RNase protection assay (RPA), we found a significant reduction in GAP-43 mRNA in the DLPFC, but not in the hippocampus, of patients with schizophrenia. With *in situ* hybridization histochemistry (ISHH), performed on a separate cohort, we confirmed the reduction of GAP-43 mRNA in the DLPFC of patients with schizophrenia. We detected reduced GAP-43 mRNA per neuron in layers III, V and VI of patients with schizophrenia compared with normal controls and patients with bipolar disorder. Thus, glutamate neurons in DLPFC of schizophrenic patients may synthesize less GAP-43, which could reflect fewer and/or less modifiable connections than those in normal human brain, and which may be consistent with the deficits of prefrontal cortical function that characterize schizophrenia.

Introduction

There is considerable evidence that schizophrenia is associated with anatomical and functional abnormalities of the dorsolateral prefrontal cortex (DLPFC) (Goldman-Rakic, 1991; Weinberger, 1993; Lewis, 1997; Weickert and Kleinman, 1998). Clinically, the cognitive problems of patients with schizophrenia resemble those of patients with prefrontal brain lesions and include reduced mental f lexibility and working-memory deficits [reviewed by Goldberg and Gold (Goldberg and Gold, 1995)] (Goldman-Rakic, 1991, 1994). Compared with healthy individuals, patients with schizophrenia have reduced blood f low in DLPFC when performing executive cognitive tasks that normally increase blood f low to DLPFC (Weinberger *et al.*, 1986; Andreasen *et al.*, 1992; Ganguli *et al.*, 1997). While the precise anatomical correlates of these behavioral and functional abnormalities are unknown, recent brain-imaging studies suggest that the integrity of cortical neurons and/or their connections may be altered (Weinberger *et al.*, 1992; Frith *et al.*, 1995; Bertolino *et al.*, 1996, 1998). Increases in neuronal cell density in Brodmann's areas 9 and 46 (Selemon *et al.*, 1995, 1998), along with decreases in DLPFC synaptic associated proteins (Glantz and Lewis, 1997; Thompson *et al.*, 1998; Honer *et al.*, 1999; Karson *et al.*, 1999), in patients with schizophrenia suggest that reduced neuronal connections or reduced neuropil may underlie functional abnormalities in the DLPFC of patients with schizophrenia.

The formation and maintenance of neuronal connections are complex processes involving the regulation of many genes and proteins. One such protein, growth-associated protein 43 (GAP-43), is a neuron-specific phosphoprotein localized to the presynaptic membrane and is a substrate for protein kinase C [reviewed by Benowitz and Routtenberg (Benowitz and Routtenberg, 1997)]. Manipulations that abolish GAP-43 expression result in disruption of axon outgrowth and can lead to premature death of the organism (Shea *et al.*, 1991; Aigner and Caroni, 1993, 1995; Shea and Benowitz, 1995; Strittmatter *et al.*, 1995), whereas transgenic overexpression of GAP-43 results in the spontaneous formation of new synapses (Zuber *et al.*, 1989; Aigner and Caroni, 1995). In developing rodent brain, GAP-43 expression is high during times of axon elongation (Jacobson *et al.*, 1986), but declines as the animals mature. In humans, GAP-43 messenger ribonucleic acid (mRNA) and protein are found in embryonic telencephalon during periods of differentiation and axon extension (Honig *et al.*, 1996; Kanazir *et al.*, 1996). Thus, GAP-43 may be integral to neurite outgrowth in both rodents and humans.

The function of GAP-43 in the adult brain is less well understood, but studies in rodents demonstrate that GAP-43 may mediate experience-dependent 'plasticity' and long-term potentiation (Nelson and Routtenberg, 1985; Lovinger *et al.*, 1986). Levels of GAP-43 vary according to the region of primate brain examined and roughly correlate with the level of functional complexity associated with a particular cortical region (Nelson *et al.*, 1987). In the adult human brain, GAP-43 mRNA and protein are abundant in neocortical areas associated with high degrees of behavioral 'plasticity' and learning, including the prefrontal cortex, and less abundant in neocortical primary sensory areas (Neve *et al.*, 1987; Neve *et al.*, 1988; Benowitz *et al.*, 1989). In adults, GAP-43 protein may function to enhance both growth and retraction of presynaptic terminals in cortical brain areas.

Although the anatomical and molecular substrates of behavioral change in humans are unknown, it has been proposed that the functional 'plasticity' of DLPFC may depend on maintenance of proper connections between prefrontal regions and other cortical and subcortical structures (Weinberger, 1993). In primates, the granular prefrontal cortex is interconnected with multi-modal association areas in the inferior parietal, superior temporal and orbital prefrontal regions (Nauta, 1971; Fuster *et al.*, 1985), the head of the caudate nucleus and the mediodorsal nucleus of the thalamus (Arikuni *et al.*, 1983; Yeterian and Pandya, 1994). Proteins that are found in the presynaptic terminals of these projections, such as GAP-43, are synthesized in the cell bodies in the DLPFC and are anterogradely transported to terminals at target sites within the prefrontal cortex and distant from the prefrontal cortex. As a correlate of the proposed abnormal DLPFC neuronal connectivity and possible synaptic pathology in the DLPFC of patients with schizophrenia, we hypothesized that GAP-43 mRNA levels would be reduced.

Table 1
Demographic information on Cohort 1

Case no.	Diagnosis	Age/sex	Race	Side	pH	PMI (h)	Months in freezer	COD	MOD	Toxicology
1	CON	53/f	B	R	5.76	22	96	burns	pending	n.a.
2	CON	34/m	B	L	6.60	37	106	ASCVD	natural	blood EtOH 0.06%,
3	CON	68/m	B	L	6.57	20	113	ASCVD	natural	n.a.
4	CON	47/m	B	R	6.62	57	118	MI	natural	n.a.
5	CON	47/m	B	R	6.54	24.5	119	GSW to chest	homicide	blood EtOH 0.10%,
6	CON	58/f	B	L	6.54	26.5	147	ASCVD	natural	negative
7	CON	39/f	B	L	6.34	41.5	147	ASCVD	natural	negative
8	CON	40/m	W	L	6.44	48.5	83	ASCVD	natural	negative
9	CON	46/f	B	L	5.93	21.5	68	cardiomyopathy	natural	negative
10	CON	74/m	W	L	6.65	33.5	80	stab wounds to chest	homicide	blood EtOH 0.04%,
11	CON	45/m	W	L	6.61	17	65	crushing injury to chest	accident	n.a.
12	CON	47/m	B	L	6.03	59.5	78	acute bronchial asthma	natural	blood morphine 0.015 mg/dl
13	CON	46/m	W	L	6.71	29	70	ASCVD	natural	negative
14	CON	55/m	B	R	6.00	10.5	67	MI (ASCVD)	natural	lidocaine 'detected'
15	CON	48/f	B	R	6.08	18.5	66	pulmonary artery thrombosis	natural	phensuximide 'detected'
16	CON	60/f	W	L	6.40	10	66	ASCVD	natural	lidocaine 'detected'
17	CON	61/f	B	R	6.15	63.5	63	multiple blunt force injuries	accident	n.a.
Mean (SD)		51 (11)			6.35 (0.30)	31.8 (16.9)	91.3 (28.6)			
18	CUS	53/f	B	R	6.35	27.5	107	ASCVD	natural	lidocaine & thioridazine 'detected'
19	CUS/TD	71/f	W	L	6.41	47.5	103	ASCVD	natural	phenothiazine metabolites 'detected'
20	CDS	36/f	B	L	6.33	60	109	acute peritonitis (ruptured appendix)	natural	negative
21	CUS	48/m	B	R	6.42	48.5	103	ASCVD	natural	carbamazepine 'detected'
22	CDS	48/m	B	L	5.70	40.5	107	internal obstruction (volvulus of colon)	natural	blood acetone 20 mg
23	CUS	36/m	B	R	6.56	13	97	blunt force injuries (fall)	suicide	phenothiazine metabolites 'detected'
24	CUS	46/m	B	R	6.35	24.5	42	ASCVD	natural	lidocaine & benztopine 'detected'
25	CPS/TD	44/f	B	R	6.51	36.5	95	cardiomegaly (hypertension)	natural	haloperidol & clonidine 'detected'
26	CPS	26/m	H	R	6.76	14.5	81	asphyxia (hanging)	suicide	n.a.
27	CUS	71/f	W	L	6.63	32.5	113	drowning	accident	thioridazine 'detected'
28	CUS	46/m	B	R	6.73	25	116	blunt force injuries (fall)	suicide	haloperidol & diphenhydramine 'detected'
29	CPS	45/m	H	L	6.15	30	112	ASCVD	natural	negative
30	CUS	34/m	B	R	6.50	23	80	acute benztropine intoxication	undetermined	blood EtOH 0.10%,
31	CUS	54/m	B	R	6.31	24	67	subarachnoid hemorrhage	natural	blood EtOH 0.32%, benztropine & doxepin 'detected'
32	CUS	80/f	W	L	6.52	59.5	63	ASCVD (aspiration of food)	natural	negative
33	CUS	48/m	W	R	6.29	15	64	dilutional hyponatremia (hypo-osmolar coma)	natural	lidocaine 'detected'
34	CUS	59/f	W	L	6.54	7.5	118	pulmonary embolism	natural	n.a.
35	CUS	49/f	B	R	6.23	15	39	COPD	natural	negative
36	CUS	34/m	B	R	6.23	36.5	68	acute benztropine intoxication	undetermined	benztropine 'detected'
37	CUS/TD	64/f	B	R	6.48	20.5	71	asphyxia (aspiration)	accident	negative
38	CPS	34/f	B	R	6.28	84	109	overdose (tricyclics)	suicide	amitriptyline & nortriptyline 'detected'
Mean (SD)		49 (14)			6.39 (0.23)	32.6 (18.9)	88.8 (24.5)			

Means and standard deviations are printed below the last individual in each group. Abbreviations are as follows: PMI = post-mortem interval, COD = cause of death, MOD = manner of death, CON = normal control, CUS = chronic undifferentiated schizophrenia, CDS = chronic disorganized schizophrenia, CPS = chronic paranoid schizophrenia, TD = tardive dyskinesa, m = male, f = female. B = Black, W = White, H = Hispanic, R = right, L = left, ASCVD = arteriosclerotic cardiovascular disease, GSW = gunshot wound, MI = myocardial infarction, COPD = chronic obstructive pulmonary disease, n.a. = not available.

Materials and Methods

Brain Collection

Cohort 1

Cohort 1, used in the RNase protection assay (RPA), included 21 patients with schizophrenia and 17 normal controls (Table 1) matched for age, sex, race, brain pH, post-mortem interval (PMI, defined as time between death and brain freezing) and time in the freezer (defined as the number of months between brain freezing and RNA extraction). No statistical differences among the groups for the demographic variables were detected. Post-mortem brains were collected at the Clinical Brain Disorders Branch (NIMH, St Elizabeths) as previously described (Kleinman *et al.*, 1995). Briefly, 1.5 cm coronal slabs through the entire cerebrum of each human brain were rapidly frozen in a pre-chilled dry-ice isopentane slurry bath and stored at –80°C. The time the tissue was stored at –80°C, before RNA was extracted (freezer time), was not

significantly different between the brains of normal individuals (91.3 ± 28.6 months, mean ± SD) and those with schizophrenia (88.8 ± 24.5 months). The number of psychiatric charts available for review varied according to duration of illness (26.5 ± 15.2 years) and typically amounted to >1000 pages of clinical information. Diagnosis was determined by independent reviews of clinical records by two board certified psychiatrists who used the Diagnostic Evaluation After Death (Zalcman *et al.*, 1983; Keks *et al.*, 1999) as a guide to review the material available on each case. Cases that met DSM-IV criteria for schizophrenia were used in our study (American Psychiatric Association, 1994). Disagreements between the two independent reviews were resolved by requesting a third psychiatrist's review of the case. Out of the 21 patients in Cohort 1 who were diagnosed with schizophrenia, 15 patients were of the chronic undifferentiated subtype, 2 patients of the chronic disorganized subtype and 4 patients of the chronic paranoid subtype. The average age of disease onset was 22.4 ± 5.2 years of age (Table 2). All patients diagnosed with schizophrenia in Cohort 1 had documented auditory hallucinations.

Table 2

Clincal information on patients with schizophrenia and bipolar disorder in Cohort 1 and Cohort 2

Case	Age of onset	Illness duration	Psychiatric medications at death (mg PO)	Last CPZ equivalent	Daily CPZ equivalent	Lifetime CPZ (mg/eq)
18	24	29	thioridazine 200 TID, lithium 600 hs	600	250	2.6×10^6
19	15	56	mesoridazine 25-50 BID	100	500	0.6×10^6
20	23	13	thiothixene, lithium, benztropine mesylate	n.a.	n.a.	n.a.
21	22	26	haloperidol 25 QID, carbamazepine 400 BID, benztropine mesylate 2 BID	2000	275	2.6×10^6
22	19	29	haloperidol 45 mg po qd	900	533	5.6×10^6
23	21	16	fluphenazine 20 qd-wk	400	850	5.0×10^6
24	23	23	fluphenazine decanoate i.m., lithium 300 qd, benztropine mesylate 2 qd	n.a.	n.a.	n.a.
25	29	15	haloperidol 5 BID	200	200	1.1×10^6
26	17	9	chlorpromazine 50 qhs	50	450	1.5×10^6
27	22	49	mesoridazine 300 qd	400	650	7.1×10^6
28	36	10	haloperidol 5 TID	300	300	1.1×10^6
29	20	25	fluphenazine 10 qd, benztropine mesylate 2 BID	200	900	8.2×10^6
30	19	15	fluphenazine 40 qd, lithium 1200 qd, benztropine mesylate 8 qd, doxepine 50 qhs	800	700	3.8×10^6
31	23	31	fluphenazine decanoate 50 mq wk	2400	800	9.1×10^6
32	24	56	haloperidol 10 TID, benztropine mesylate 2 BID	600	600	7.0×10^6
33	33	15	chlorpromazine 300 qd, carbamazepine 400 qd, diphenhydramine 50 qd	300	300	1.6×10^6
34	19	40	chlorpromazine 100 BID	200	200	2.2×10^6
35	17	32	fluphenazine 30 qd, lithium 900 qd	600	600	7.0×10^6
36	26	8	thiothixene, amitryptiline, benztropine mesylate	n.a.	n.a.	n.a.
37	19	45	haloperidol decanoate 50 i.m. q 4 wks, haloperidol 5 TID, clonazepam 2 TID	900	400	5.3×10^6
38	19	15	fluphenazine decanoate 37.5 i.m. wk, benztropine mesylate (dose n.a.)	1800	1000	5.5×10^6
Mean (SD)	22.4 (5.2)	26.5 (15.2)		708 (683)	528 (252)	4.3×10^6
45	20	17	none (untreated for 20 years)			0.18×10^6
46	27	33	thioridazine, amitryptiline			1.6×10^6
47	38	24	none (untreated for several months)			1.0×10^6
48	13	17	risperidone			1.0×10^6
49	18	13	clozapine			0.08×10^6
50	15	45	none (never given)			0
Mean (SD)	21.8 (9.3)	24.8 (12.1)				0.64×10^6
51	22	7	risperidone, lithium			0.18×10^6
52	39	14	lithium, bupropion, clonazepam, lorazepam			0.05×10^6
53	30	27	haloperidol, diphenhydramine			1.2×10^6
54	19	15	risperidone, valproate, venlafaxine			0.14×10^6
55	19	6	thiothixene, carbamazepine, lithium, trazadone			0.15×10^6
Mean (SD)	25.8 (8.7)	13.8 (8.4)				0.34×10^6

Ages of onset and illness durations are given in years. Abbreviations are as follows: CPZ = chlorpromazine, mg/eq = milligram equivalent.

All but one patient had documented paranoid delusions; 18 patients showed evidence of thought disorder, and another 18 exhibited negative symptoms. IQ data were available for 14/21 patients (full-scale IQ for these 14 patients = 81 ± 20, mean $\pm$ SD). The total dose of neuroleptic medication given to the patients was calculated by adding the various daily medication levels as determined from available medical records and converting these levels to chlorpromazine (CPZ) equivalents as previously formulated (Torrey, 1983). A median value of drug dosage was then calculated from the CPZ equivalents to give the estimated average daily dose; this value was multiplied by the duration of illness (estimated from the earliest age of definable symptoms or age at first hospitalization) to give the estimated lifetime CPZ equivalents (Table 2).

Characterization of Normal Controls

Cases were screened by police and/or by telephone interviews of family members for a history of medical and/or psychiatric problems, including alcohol abuse and illicit drug use. Any positive history of a psychiatric problem or excessive alcohol or drug use led to the exclusion of that case from the normal control group. In addition, 80% of all cases had toxicological screens of blood. If the medical examiner believed that the index of suspicion for illicit drugs or alcohol abuse was low, a toxicological screen was not performed.

All brains (both normal controls and patients with schizophrenia) were screened for signs of macroscopic pathology at the time of autopsy; brain sections were examined microscopically with the use of Bielschowsky's silver stain on multiple cerebral areas to exclude the presence of neuritic pathology as seen in Alzheimer's disease (AD). Those cases with an unclear psychiatric diagnosis, evidence of cocaine or PCP abuse (history and/or toxicology), cerebrovascular disease, autolysis, subdural hematoma, neuritic pathology or other pathological features were excluded from the analysis.

Cohort 2

The brains composing Cohort 2, used in the *in situ* hybridization histochemistry (ISHH), were obtained from the Stanley Foundation Brain Bank and included six normal controls, six patients with schizophrenia and five patients with bipolar disease who received neuroleptics. The brains in Cohort 2 were collected, evaluated and screened in the same manner as those in Cohort 1 (Table 3). For Cohort 2, five out of the six individuals diagnosed with schizophrenia were of the chronic undifferentiated type and one individual was of the chronic paranoid type. The average age of disease onset was 21.8 ± 9.3 years of age for the patients with schizophrenia and 24.2 ± 8.6 years of age for the patients with bipolar disorder. The three groups in Cohort 2 were matched as to PMI, brain pH and postnatal age. No statistical differences among the groups for the demographic variables were detected (Table 3).

pH Determinations

The brain tissue pH was determined from homogenized cerebellar tissue; in <5% of the cases, frontal cortex was used. Tissue (0.4–0.8 g) was thawed and homogenized in 10 times the tissue volume in double-distilled (dd) H_2O (pH 7.0) and the homogenate pH was measured with a Sentron pH meter. The pH electrode was washed thoroughly in ddH_2O (pH 7.0) after each sample and recalibrated after every 10 samples.

Table 3
Demographic information on Cohort 2

Case	Diagnosis	Age/sex	Race	Side	pH	PMI (h)	Months in freezer	COD	MOD
39	CON	59/m	W	L	6.4	26	8	ASCVD	natural
40	CON	34/m	W	R	6.3	23	8	ASCVD	natural
41	CON	48/m	W	L	6.2	17	10	ASCVD	natural
42	CON	18/m	W	R	6.3	53	14	multiple injuries, transected spinal cord (T6)	accident
43	CON	48/m	H	R	6.1	12	9	ASCVD	natural
44	CON	52/m	W	R	6.5	28	12	ASCVD	natural
Mean (SD)		43 (15)			6.3 (0.1)	26.5 (14.2)	10.2 (2.4)		
45	CUS	52/m	W	R	6.0	61	17	ASCVD	natural
46	CUS	60/m	W	L	6.2	31	9	asphyxiation (drowning)	accident
47	CPS	62/f	A	R	6.1	26	13	multiple blunt force injuries	accident
48	CUS	30m	W	L	5.8	32	14	bronchopneumonia	natural
49	CUS	31/m	W	R	5.8	14	7	blunt force injuries (fall)	suicide
50	CUS	60/f	W	R	6.2	40	13	ASCVD	natural
Mean (SD)		49 (15)			6.0 (0.2)	34 (15.8)	12.2 (3.6)		
51	B w/P	29/m	W	L	6.0	48	9	blunt force injuries (fall)	suicide
52	B w/o P	54/m	W	L	5.8	39	12	blunt force injuries to head (fall), subdural hematoma	accident
53	B w/ P	57/m	W	R	6.2	19	8	ASCVD	natural
54	B w/ P	34/m	W	L	6.3	23	8	blunt force injuries (jump)	suicide
55	B w/ P	25/f	W	L	6.1	24	13	hanging	suicide
Mean (SD)		40(15)			6.1 (0.2)	30.6 (12.3)	10 (2.3)		

Means and standard deviations are printed below the last individual in each group. Abbreviations not given for Table 1: B w/P = bipolar disorder with psychotic features, B w/oP = bipolar disorder without psychotic features, A=Asian.

RNA Extractions

Tissue was excised from either the right or left side of the middle third of the superior or middle frontal gyrus immediately anterior to the genu of the corpus callosum. Hippocampal tissue was dissected from along the temporal horns of the lateral ventricle from either the right or left temporal lobe. Approximately 400 mg of frozen pulverized human brain tissue from each case was assigned an RNA isolation number, weighed frozen and transferred into a pre-chilled, DEPC-treated, glass homogenizer. Each sample was homogenized in lysis buffer, layered over a cushion buffer with a higher sucrose concentration and centrifuged at 5000 g for 20 min at 4°C as described previously (Jakubowski and Roberts, 1992). The cytoplasmic RNA was further purified by proteinase K digestions and phenol:chloroform extractions (Jakubowski and Roberts, 1992). A spectrophotometer was used to quantitate the total RNA (μg) in each sample, and the integrity of ribosomal RNA bands was verified by agarose gel electrophoresis for each sample. Reisolation of RNA was performed as necessary. Total RNA was aliquoted, dried down and stored in 30 μl of hybridization buffer (80% deionized formamide, 40 mM PIPES, pH 6.7, 400 mM NaCl, 1 mM EDTA) at –80°C.

Northern Blot and RPA

The human GAP-43 DNA template used for the preparation of riboprobes corresponded to base pairs 54–832 of the cDNA (Kosik *et al.*, 1988) (gene bank XM25667), and was constructed by J.E. Cheetham, of the University of Rochester (Callahan *et al.*, 1994). Northern blot analysis was used to confirm the specificity of the GAP-43 probe for a 1.6 kB transcript in human cortical RNA (Kosik *et al.*, 1988). A ^{32}P-labeled riboprobe with a sp. act. of 1×10^9 c.p.m./μg was synthesized from the GAP-43 cDNA template. An RNA blot containing cortical regions from adult human brain (cat. no. 7755-1, Clonetech, Palo Alto, CA) was pre-hybridized in buffer containing 12.5 M formamide, 50 μg/ml salmon sperm DNA, 0.025 M KPO$_4$, 5× Denhardt's solution and 5× saline sodium citrate (SSC) in RNase-free water for 2 h at 42°C. Radioactive probe at 5 ng probe/ml was added to the hybridization buffer (prepared as above, with the addition of 10% Dextran sulfate) and the mixture was pre-equilibrated to 42°C, added to the blot and allowed to hybridize overnight at 42°C. The blot was rinsed three times in 1× SSC/0.1%SDS at room temperature (15 min), three times in 0.25× SSC/0.1%SDS at 55°C (15 min) and three times in 0.2× SSC/0.1%SDS at 68°C (15 min). The blot was air-dried and exposed to BioMax film (Kodak, Rochester, NY) overnight.

On the day of the RPA, sample tubes containing the RNA were removed from the freezer, thawed and reassigned random sample numbers using a random number table, by an individual not performing the assay. The experimenters were blind to diagnosis during sample handling and the samples were processed in a random order. RPAs were preformed as previously described (Jakubowski and Roberts, 1992; Lazar and Blum, 1992; Weickert and Blum, 1995). Riboprobes were labeled to a sp. act. of 1×10^9 c.p.m./μg RNA with [^{32}P]UTP (Amersham, Arlington Heights, IL), purified by ethanol precipitation and added (1 μl of 200 pg/μl) to the samples of 2 or 4 μg of total RNA. Standard curves for the GAP-43 RPA were generated by adding increasing amounts of *in vitro* transcribed sense strand GAP-43 RNA ranging from 0.1 pg (0.36 amol) to 100 pg (363.63 amol), and by normalizing the total RNA levels with yeast total RNA. A tube containing no sense strand (0 pg) but with total yeast RNA was included as a control to ensure that the digestion of radiolabeled antisense probe was complete. Two or four micrograms of total yeast RNA were added to the tubes containing the standards so the total amount of RNA was equivalent to the samples. Hybridization of the riboprobe to standards and samples was allowed to proceed in a 45°C water bath overnight. For RNase digestion of non-protected fragments of RNA, RNase A (10 μg/ml) and RNase T1 (2 μg/ml) were used. The protected hybrids were purified by phenol:chloroform extractions and ethanol precipitations, and were size separated on a 5% acrylamide gel. The gels were dried and exposed to autoradiographic film. The most prominent band corresponding to the appropriate size protected fragment for the standard curves and the samples were cut from the dried gels and placed in a scintillation counter. A regression analysis using known amounts of plus strand GAP-43 mRNA was used to determine the amount of GAP-43 mRNA in the samples. Aliquots from the same RNA samples were also assayed for three constitutively expressed genes, cyclophilin, β-actin and glyceraldehyde-3-phosphate-dehydrogenase (GADPH), to address potential systematic differences in RNA integrity between patients and controls that might be related to agonal state, post-mortem handling, tissue processing or RNA isolation procedures. Human cDNA templates for cyclophilin, β-actin and GADPH were purchased from Ambion (Austin, TX) as linearized triple-script plasmids. The RPAs were performed twice for both cyclophilin and GAP-43 mRNA with similar results each time; therefore the average value of mRNA from the two assays was used in the final statistical analysis.

In situ HybriDIZATION and Quantitation

Fixation, acetylation, delipidation and dehydration of the slides containing 14-μm-thick sections of frontal cortex were performed as previously described (Whitfield *et al.*, 1990). Two hundred microliters of hybridization buffer containing the [^{35}S]UTP-labeled GAP-43 riboprobe (5 ng/ml) was added to each section and hybridization was allowed to occur at 55°C overnight in humidified chambers. After the *in situ* hybridization procedure, slides, along with ^{14}C standards (American Radiolabeled Chemicals, Inc., St Louis, MO), were exposed to Kodak autoradiographic film (X-OMAT) for 3 days. With the aid of a microscope, the boundary of Brodmann's area 46 (BA 46) was delineated on Nissl-stained sections, applying the criteria described previously (Rajkowska and Goldman-Rakic, 1995). The criteria used can be outlined as follows: (i) the presence of a well defined granular layer IV; (ii) the columnar arrangement of pyramidal neurons in layer III; (iii) the increase in size of pyramidal neurons in layer III with an increase in cortical depth; and (iv) the presence of a clear transition from layer VI into the white matter. Sampling was done in BA 46 where the laminae were running parallel to the pial surface. Quantitation of optical density from the film was done blind to diagnosis (with numbers assigned by the Stanley Foundation) with the aid of NIH Image (Rasband, NIH) and Excel (Microsoft) software programs. For each slide, three lines of 170 μm width, traversing the entire cortical gray matter, were drawn perpendicular to the pial surface. Optical density, interpolated along the ^{14}C standard curve, was sampled at 85 μm pixel intervals along these lines. These data were used to construct profile plots of μCi/g of GAP-43 mRNA as it varied with cortical gray matter depth. The profiles along each line were then linearly interpolated to a common anatomical scale in units of percent cortical depth. Two sections per case were analyzed. For each case, the μCi/g of GAP-43 averaged from the six sampling lines was used in the statistical analysis, so that each individual had only one representative measurement at each cortical depth. Measurements for the representative laminae were taken from within the borders of each layer, based on percent cortical depth for each layer. These measurements were derived from average values for laminar boundaries described elsewhere (Rajkowska and Goldman-Rakic, 1995). Layer III was considered to be at 20–46% of the total cortical depth, layer V at 54–70%, and layer VI at 74–84%. Each case was analyzed independently by two neuroscientists with significantly correlated results (intraclass correlation coefficient, ICC = 0.69, $P < 0.001$).

Silver Grain Analysis

In order to determine whether GAP-43 mRNA reductions in the DLPFC of patients with schizophrenia were due to a decrease in the amount of GAP-43 mRNA per neuron, we counted the number of silver grains overlying pyramidal neurons. We chose to focus on pyramidal neurons because GAP-43 mRNA is distributed preferentially over large pyramid-shaped cells. While smaller non-pyramid-shaped neurons of the human DLPFC occasionally appeared to have positive hybridization signal and thus may have contained GAP-43 mRNA, the many smaller, more round-shaped cells appeared unlabeled and were not analyzed. Additionally, cells with a small, darkly stained nucleus with little cytoplasmic stain (possible astrocytes) did not appear to express GAP-43 mRNA in any of the groups.

Slides were dipped in NT-B2 emulsion (Kodak), stored in light-tight boxes in the dark for 9 days and then developed in D-19 developer, dehydrated and lightly stained with a Nissl counterstain. Silver grain analysis was conducted blind to diagnosis on a Zeiss Axiophot microscope equipped with a video camera and Bioquant image analysis system. We counted grains in layers III, V and VI, because it was in these layers that we had the most robust signal on the autoradiographs. Neurons in layers III, V and VI from each case were identified under bright field by their large cell size, light Nissl stain and triangular (layers III, V and VI) or fusiform shape (layer VI). The shape of the cells could be outlined by combining demarcation of cells in the light Nissl stain and GAP-43 mRNA silver grain distribution, which often extended into apical dendrites. Circles of 35 μm diameter were drawn, centered over every large triangular or fusiform-shaped neuron in a field including ~5–6 neurons. The illumination was then switched to dark field and the silver grains overlying a neuron, within the boundaries of this circle, were counted with the aid of a grain-counting macro-program written with Bioquant

software. The threshold for determining positive grains was set using the numerical value of 118–255 in the green measurement window, and was held constant for every case and for all sampling fields within each case. On average, seven microscopic fields per layer (at a final magnification of 40×) were examined and only those neurons containing numbers of silver grains fourfold above background (criteria as set in Akbarian *et al.*, 1995) were entered into the quantitative analysis. Background was determined by averaging the number of grains in three 35-μm-diameter circles over areas of gray matter neuropil from within fields in which neurons were being sampled. The background level of grains was determined separately for each case. Overall, >95% of neurons counted contained enough silver grains to reach inclusion criteria, and this percentage was similar in all three diagnostic groups. At least 30 neurons within each layer for each case (a total of 1620 neurons) were sampled within layers III, V and VI from contiguous fields using an arbitrary start point within BA 46. The average number of grains per cell for each layer for each case was used in the statistical analysis.

Statistical Analysis

The Spearman rank order correlations were run between measurements of RNA and brain cohort characteristics (Spearman *R*, Statistica, Statsoft, Tulsa, OK). For the RPAs, the data were analyzed by ANCOVA, where diagnosis or diagnosis and hemisphere were the independent factors, GAP-43 mRNA and cyclophlin mRNA were the dependent factors, and factors that correlated with GAP-43 mRNA levels were co-varied for. For the data derived from the ISHH, there were three GAP-43 measurements per case, for layers III, V and VI respectively. The average μCi/g for each layer was used in a MANOVA (Statistica), where diagnosis was the in-dependent variable and the GAP-43 mRNA in layers III, V and VI were the dependent variables. When a significant overall main effect of diagnosis was found in the MANOVA, the groups were compared by post-hoc *t*-tests run separately for layers III, V and VI.

Results

RNA

The total yield of RNA extracted from the DLPFC did not significantly differ between the normal controls (0.15 ± 0.08 μg RNA/mg tissue, mean ± SD) and the patients with schizophrenia (0.19 ± 0.10 μg RNA/mg tissue, mean ± SD) in Cohort 1 ($t = -1.30$, df = 36, $P = 0.20$). No qualitative relationship between integrity of ribosomal RNA bands and PMI was noted and no significant correlation between PMI or freezer storage time and total yield of RNA was detected ($R = -0.01$ and 0.02, respect-ively). This is in agreement with other reports of general RNA stability in human brain tissue over a wide range of post-mortem intervals (Johnson *et al.*, 1986; Barton *et al.*, 1993). Total RNA and pH measurements were positively correlated ($R = 0.37$, $P < 0.05$, pH range 5.70–6.76), suggesting that brain pH measure-ments may be predictive of overall RNA stability as reported previously (Harrison *et al.*, 1995). We were unable to detect any significant correlation between yield of total RNA from pre-frontal cortical tissue and age of subject ($R = -0.14$, $P = 0.42$, age range 20–80 years). PMI and brain tissue pH were not correlated ($R = 0.05$, $P = 0.75$).

Northern Blot

The GAP-43 riboprobe used in our experiments recognized one major band at the expected 1.6 kB transcript size in RNA extracted from human brain (Fig. 1). Additionally, GAP-43 mRNA levels were lowest in medulla and spinal cord (lanes 3 and 4), intermediate in cerebellum and putamen (lanes 1 and 8) and highest in cortical regions, including the occipital cortex (lane 5), frontal cortex (lane 6) and temporal cortex (lane 7).

GAP-43 mRNA RPA

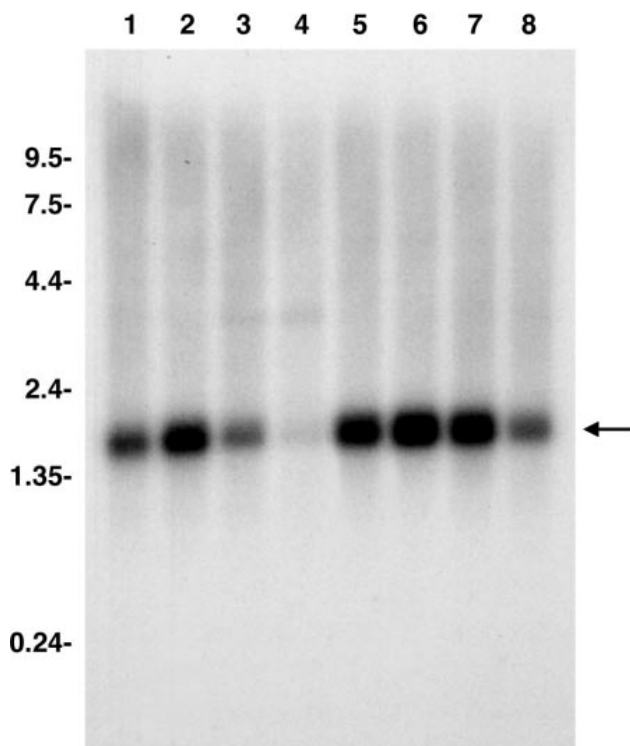The autogradiographic films generated from the RNase pro-

Figure 1. Northern hybridization of GAP-43 ^{32}P-labeled probe to cortical poly-A RNA extracted from human brain showing one major 1.6 kB band (arrow) in lanes 1-8. Lanes contain poly(A) mRNA extracted from the pooled right and left hemispheres from multiple normal adult individuals from the following regions (1) whole cerebellum; (2) whole cerebral cortex; (3) medulla; (4) spinal cord; (5) occipital pole; (6) frontal lobe; (7) temporal lobe; (8) putamen.

Figure 2. Autoradiographic film produced from the RNase protection assay. The black bar on the left denotes the migration of a 622 bp and a 527 bp piece of the *Msp*1-digested pBR322 marker DNA (M). The standard curve (top) generated with known amounts of *in vitro* transcribed sense strand GAP-43 mRNA ranges from 0 to 100 pg (left to right) and is followed by the 38 samples which showed a 778 bp protected GAP-43 mRNA fragment (N = normal control, S = schizophrenic).

Figure 3. Mean RNA levels in the prefrontal cortex of normal controls and schizophrenics from the RNase protection assays: attomoles of GAP-43/μg total cytoplasmic RNA (A) and of cyclophilin/μg total cytoplasmic RNA (B). Error bars represent the standard error. The mean GAP-43 mRNA level was 14.43 ± 10.68 amol in patients with schizophrenia (S) and 23.38 ± 15.13 amol in normal controls (N) (*$P < 0.05$).

tection assays showed a protected fragment at the predicted 778 bp in all cases (Fig. 2). The radioactive signal found below the protected band in each sample lane is also found in the standard curve as is commonly seen when using RNase protection assay techniques (Lazar and Blum, 1992), and does not reflect degradation of extracted RNA. The lack of a signal in the 0 lane (which contained no input RNA from brain) shows that there was complete digestion of the radiolabeled probe. We found a 38% reduction in GAP-43 mRNA levels/μg total RNA in the DLPFC of patients with schizophrenia (mean ± SD = 14.43 ± 10.68 amol), as compared with normal controls (mean ± SD = 23.38 ± 15.13 amol) (Fig. 3A). GAP-43 mRNA levels correlated significantly with both PMI ($R = -0.32$, $P = 0.05$) and pH ($R = 0.35$, $P = 0.03$) (Fig. 4A,C). GAP-43 mRNA and postnatal age were not significantly correlated ($R = -0.02$, $P = 0.92$, Fig. 4E). An analysis of DLPFC GAP-43 mRNA levels using an ANCOVA with pH and PMI entered as covariates revealed a significant effect of diagnostic group ($F = 5.70$, df = 1,34, $P = 0.023$), where patients with schizophrenia had reduced GAP-43 mRNA compared with controls (a significant decrease of GAP-43 mRNA was also found by using a t-test, $t = 2.14$, df = 36, $P = 0.04$).

Next, we considered if brain hemisphere had a significant effect on GAP-43 mRNA levels. Using a two-way ANCOVA, there was again a significant main effect of diagnosis on GAP-43 mRNA levels ($F = 8.31$, df = 1,32, $P = 0.007$), where patients with schizophrenia had significantly less GAP-43 mRNA. GAP-43 mRNA levels were increased by 45% on the right side of the brain compared with the left for both normal individuals and patients
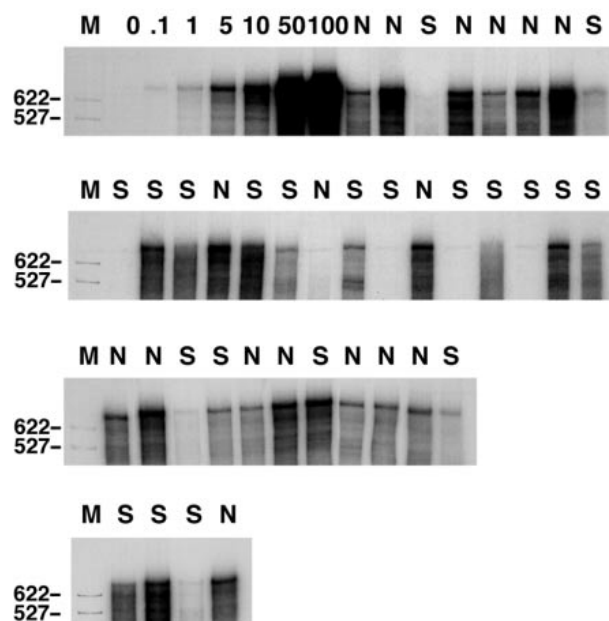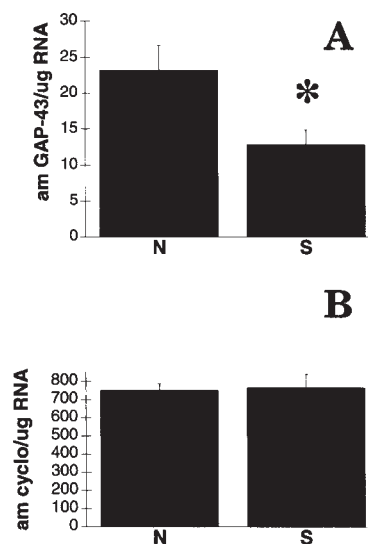
with schizophrenia; however, this effect approached, but did not reach, statistical significance ($F = 2.80$, df = 1,32, $P = 0.10$). No interaction effect between hemisphere and diagnosis was detected ($F = 1.93$, df = 1,32, $P = 0.17$).
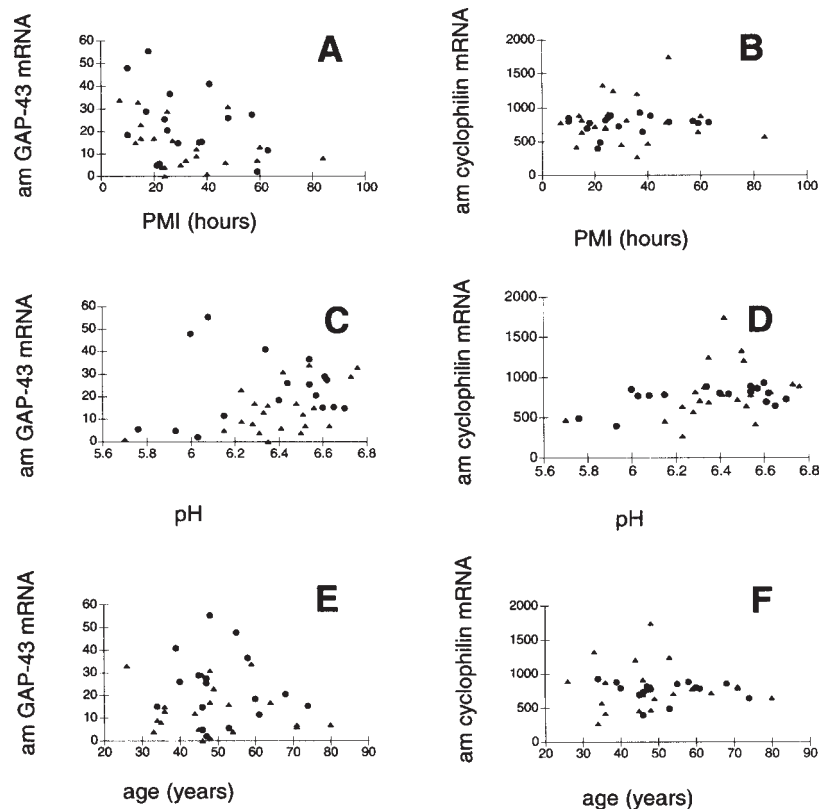
As a test of the regional specificity of the GAP-43 mRNA

Figure 4. Scattergrams of DLPFC GAP-43 mRNA in amol (*y*-axis) and PMI in hours (*x*-axis, *A*), brain pH (*x*-axis, *C*) and postnatal age in years (*x*-axis, *E*) for all 38 samples are displayed. Scattergrams of DLPFC cyclophilin mRNA in amol (*y*-axis) and PMI in hours (*x* axis, B), brain pH (*x* axis, D) and postnatal age in years (*x*-axis, *F*) for all 38 samples are shown (filled circles = normal controls, filled triangles = schizophrenics).

reduction, we used RPA to determine the GAP-43 mRNA level in total RNA extracted from hippocampus (Cohort 1) but did not detect a significant difference in GAP-43 mRNA levels between schizophrenic patients and controls (using ANCOVA with PMI and pH as covariates, $F = 0.20$, df = 1,28, $P = 0.65$). In fact, hippocampal GAP-43 mRNA was slightly (17%) higher in patients with schizophrenia compared with normal controls. This suggests that the reduction in GAP-43 mRNA in the DLPFC of patients with schizophrenia is not due to uncontrolled differences in the agonal state of patients or post-mortem brain handling, which would not be expected to have regionally selective effects.

Lastly, we tested to see whether levels of GAP-43 mRNA correlated with neuroleptic medication histories. There was a significant negative correlation between GAP-43 mRNA levels and average daily doses of medication converted to chlorpromazine equivalents, in both the DLPFC ($R = -0.60$, $P = 0.008$) and the hippocampus ($R = -0.67$, $P = 0.05$), among schizophrenic patients. GAP-43 mRNA levels were also correlated with the estimated lifetime neuroleptic dose in the DLPFC ($R = -0.47$, $P = 0.05$) and the hippocampus ($R = -0.58$, $P = 0.04$). GAP-43 mRNA levels did not correlate significantly with daily dose of medication at death (in DLPFC $R = -0.27$, $P = 0.28$; in hippocampus $R = -0.30$, $P = 0.33$) nor with the duration of illness in the DLPFC ($R = -0.16$, $P = 0.50$) or in the hippocampus ($R = -0.08$, $P = 0.76$).

Cyclophilin RNA RPA

In preliminary studies, we found that the expression of cyclo-

philin, β-actin and GADPH did not differ between patients with schizophrenia and normal controls in the DLPFC. Cyclophilin mRNA proved to have the least amount of subject-to-subject variability and was chosen for further analysis. Cyclophilin mRNA was not altered in patients with schizophrenia relative to controls (ANCOVA with PMI and pH as covariates, $F = 0.16$, df = 1,34, $P = 0.69$, Fig. 2*B*). Unlike GAP-43 mRNA, the level of cyclophilin mRNA did not correlate with PMI ($R = 0.06$, $P = 0.73$), suggesting that the effect of PMI on mRNA levels may be message selective (Fig. 4*B*). Cyclophilin mRNA levels did correlate significantly with brain pH ($R = 0.39$, $P = 0.02$, Fig. 4*D*), but not with age ($R = -0.04$, $P = 0.80$, Fig. 4*F*). We found that the ratio of GAP-43 to cyclophilin was significantly reduced in the DLPFC of patients with schizophrenia (ANCOVA with pH and PMI as covariates, $F = 5.65$, df = 1,34, $P = 0.023$).

GAP-43 mRNA In Situ HYBRIDIZATION

GAP-43 mRNA hybridization signal was primarily distributed over pyramidal neurons in both the supragranular and infragranular layers in all three diagnostic groups (Figs 5 and 7). There were very few scattered silver grains present in sections hybridized with radiolabeled GAP-43 sense strand control (Fig. 5*D*). The GAP-43 mRNA hybridization signal from the autoradiographic films was significantly reduced in the schizophrenic brains as compared with normal controls [Wilks' Lambda = 0.34 (df = 3,8), $P < 0.05$] in layers III (mean 29% reduction), V (mean 28% reduction) and VIa (mean 25% reduction, post-hoc tests, $t = 2.56$, $P = 0.01$, $t = 3.29$, $P < 0.01$ and $t = 2.16$, $P < 0.05$, respectively). In contrast, the GAP-43 mRNA
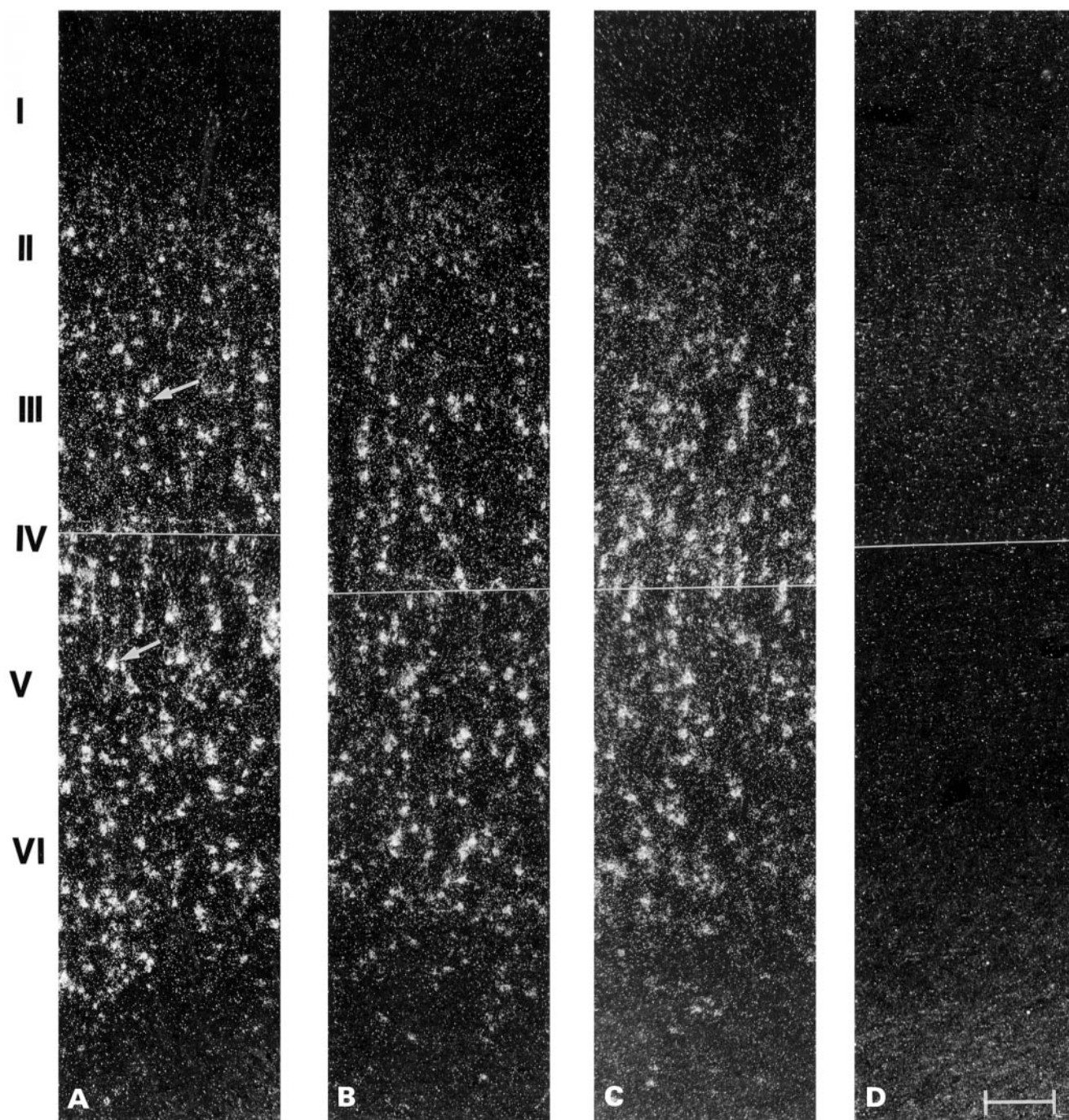
Figure 5. Darkfield photomicrograph showing hybridization signal for GAP-43 mRNA in layer III and in layers V/VI overlying pyramidal neurons (arrows) in the normal (*A*), schizophrenic (*B*) and bipolar disorder (*C*) brains. (*D*) The absence of labeling when the GAP-43 mRNA sense strand was hybridized to the sections. A relative decrease in GAP-43 mRNA hybridization signal is seen over cortical pyramidal neurons in layers III and V/VI of the schizophrenic prefrontal cortex as compared with the bipolar disorder and control subjects. (Scale bar = 200 μm).

levels in the individuals with bipolar disorder did not differ significantly from the normal controls [Wilks' Lambda = 0.834 (df = 3,7), $P = 0.72$]. In the bipolar group, the level of GAP-43 mRNA did not correlate with medication dosages in any layer (all $P > 0.10$). Quantitative analysis at the cellular level revealed that the GAP-43 silver grains per cell varied significantly in the pyramidal neurons according to diagnostic group (MANOVA, Wilks' Lambda = 0.34, df = 6, 22, $P = 0.04$, Fig. 6). Neurons

residing in layer III, V and VI of the schizophrenics had a 40, 35 and 36% reduction, respectively, in number of GAP-43 mRNA silver grains per neuron compared with controls. The reduction in GAP-43 mRNA as detected by silver grain analysis in Cohort 2 was similar in magnitude to that detected by RPA in Cohort 1 and was statistically significant in all layers examined (post-hoc *t*-tests all $P < 0.01$). When patients with schizophrenia were compared with individuals diagnosed with bipolar disorder, layers III, V

and VI showed a 29, 24 and 34% reduction, respectively, in the number of silver grains per neuron in patients with schizophrenia (Fig. 6). This reduction in GAP-43 mRNA/pyramidal neuron in the patients with schizophrenia compared with the neuroleptic control (bipolar disorder) group was significant in layers III and VI ($P < 0.05$) and approached significance in layer V ($P = 0.09$). The number of silver grains/neuron in patients with bipolar disorder was not significantly reduced compared with normal non-neuroleptic-medicated controls (all layers, $P > 0.05$). The amount of GAP-43 silver grains/neuron did not correlate
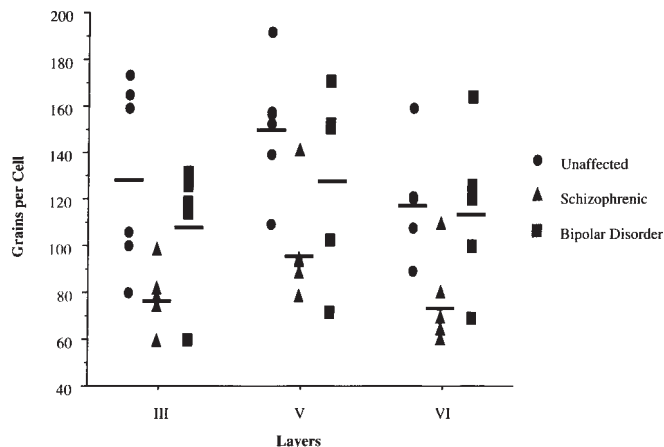
with the lifetime dose of neuroleptic medication in the patients with bipolar disorder (all layers, $P > 0.10$).

Discussion

We have found a reduction in GAP-43 mRNA levels in the DLPFC of patients with schizophrenia using two complementary techniques in two separate cohorts. Additionally, we found several factors that may contribute to post-mortem human brain DLPFC GAP-43 mRNA levels, i.e. brain pH, PMI, brain hemisphere and medication history. This may explain the high degree of subject-to-subject variability found in our study and may explain some of the inconsistencies across studies of GAP-43 in post-mortem human brain tissue. While the GAP-43 mRNA reduction may be a component of the primary disease process in schizophrenia, it is important to consider an alternative possibility; that this represents an epiphenomenon related to the experience of having schizophrenia, or exposure to neuroleptic drugs or to other events associated with a lifetime of unremitting mental illness. We have detected a relationship between average daily dose and lifetime dose of neuroleptic medication and GAP-43 mRNA levels, and this suggests that neuroleptics may affect GAP-43 mRNA levels. Alternatively, this correlation may reflect another common variable that determines both GAP-43 mRNA levels and neuroleptic dose, such as illness severity, rather than suggesting a direct causal relationship between neuroleptics and GAP-43 mRNA levels.

Several lines of data argue against neuroleptic exposure and nonspecific illness factors as the cause of the reduction of GAP-43 mRNA in the DLPFC. First, we did not observe a decrease in GAP-43 mRNA levels in the hippocampus from the same group of patients in which we found the reduction in DLPFC. Second, we did not detect a significant decrease in GAP-43 mRNA levels in the neuroleptic-treated and chronically ill bipolar disorder

Figure 6. The average number of silver grains per neuron per lamina III, V and VI are plotted for each normal control (circles), and schizophrenic (triangles) and bipolar disorder patients (squares). The number of GAP-43 mRNA silver grains per neuron is reduced in layers III, V and VI in the patients with schizophrenia compared with both control groups.
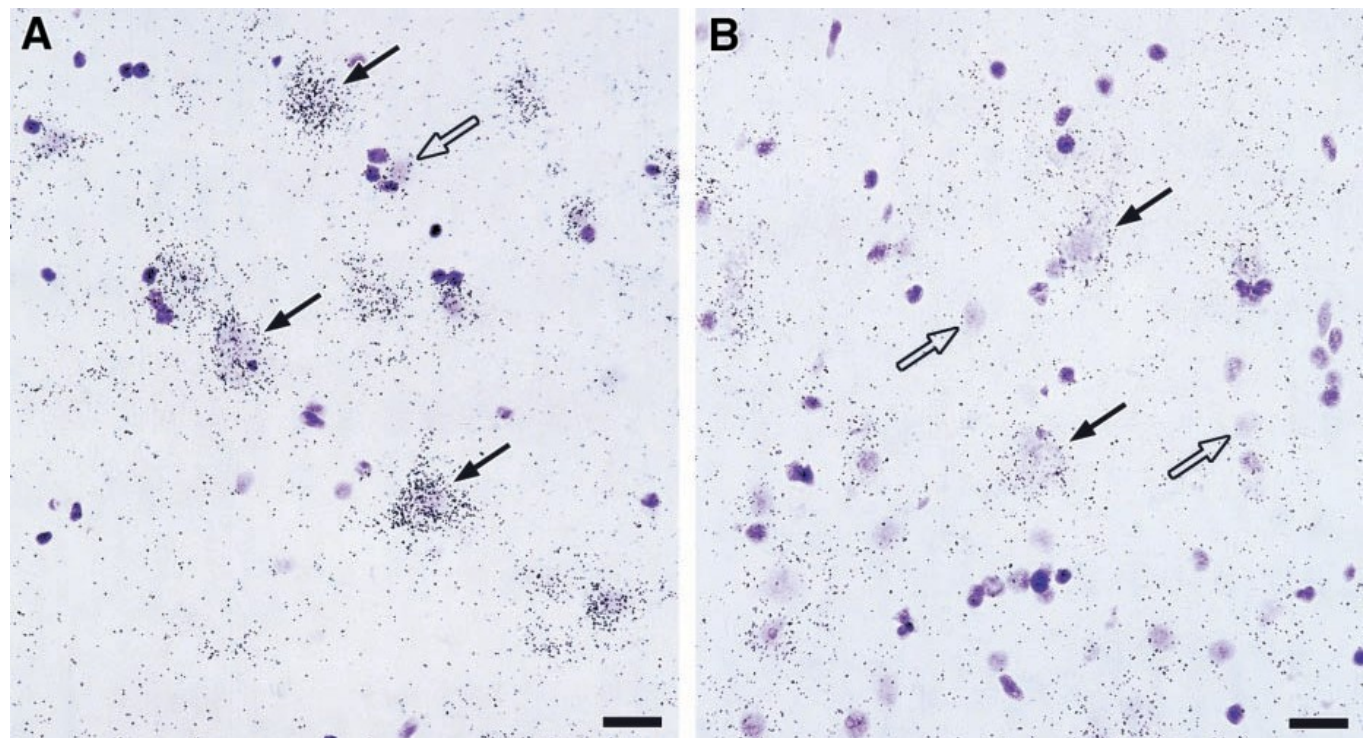

Figure 7. High power bright field (A,B) of cells in layer III are shown. Note that pyramidal neurons in the brain of normal individuals (A) and of patients with schizophrenia (B) contain GAP-43 silver grains (solid arrows in A and B). Many non-pyramidal, round cells that contain lightly Nissl-stained nuclei contain a low level of silver grains (open arrow in A and B). Small darkly Nissl-stained nuclei have no apparent silver grain clusters localized over them. (Scale bar = 20 μm).

group compared with normal controls, nor did GAP-43 mRNA levels correlate with neuroleptic exposure in the group of bipolar patients examined. Also, the amount of GAP-43 mRNA per neuron was significantly reduced in layers III and VI in individuals with schizophrenia compared with individuals with bipolar disorder. Third, chronic treatment with haloperidol does not significantly alter GAP-43 mRNA levels in the rodent neocortex (Eastwood *et al.*, 1997).

Our findings may appear inconsistent with two earlier studies of GAP-43 mRNA and protein in the DLPFC of patients with schizophrenia. A similar reduction in GAP-43 mRNA levels was found in some cortical areas (primary visual and anterior cingulate) of patients with schizophrenia, but no significant changes were found in the DLPFC (Eastwood and Harrison, 1998). Although there are no obvious reasons for the discrepancy between the Eastwood and Harrison study and our own, the differences could relate to subtle differences in cohort characteristics or brain storage conditions. Another study has reported that GAP-43 protein in brain homogenates from DLPFC (BA9) is increased; they used a fairly small number of cases (five patients with schizophrenia and four normal controls) and a quantitative study of GAP-43 mRNA levels was not conducted (Perrone-Bizzozero *et al.*, 1996). Since many axon terminals in the DLPFC arise from intrinsic sources, one may expect that changes in GAP-43 protein may be mirrored by similar changes in GAP-43 mRNA levels. However, this is not what we found. The putative increase in GAP-43 protein in DLPFC may arise from a compensatory response of afferent terminals originating in distant sites. Alternatively, the increase in GAP-43 protein in the DLPFC could result from a deficit in fast axonal transport of the GAP-43 protein (Liu *et al.*, 1991), and thus GAP-43 protein may inappropriately accumulate in DLPFC cell bodies. Further work evaluating the anatomical and subcellular localization of altered GAP-43 protein in the brains of patients with schizophrenia is necessary to explore these possibilities.

The reduction of GAP-43 mRNA reported here suggests that there may be reduced GAP-43 protein in presynaptic terminals, less phosphoprotein activity in presynaptic terminals and a diminished 'plasticity' of DLPFC pyramidal neurons in patients with schizophrenia. Layers III, V and VI in the schizophrenic DLFPC had less GAP-43 mRNA hybridization signal, which could represent fewer GAP-43 producing neurons or could reflect less GAP-43 mRNA per neuron. In support of the latter interpretation, silver grain analysis has confirmed that there were reduced levels of GAP-43 mRNA overlying pyramidal neurons in the DLPFC of patients with schizophrenia. This suggests that glutamate pyramidal neurons in the DLPC may synthesize less GAP-43 protein in patients with schizophrenia. Our data do not rule out the possibility that there may also be fewer GAP-43 producing neurons in the DLPFC of patients diagnosed with schizophrenia. Prior studies have reported normal numbers of pyramidal neurons in the DLPFC of patients with schizophrenia (Pakkenberg, 1993; Akbarian *et al.*, 1995), an increase in pyramidal cell density (Selemon *et al.*, 1995, 1998) and a smaller size of pyramidal neurons (Rajkowska *et al.*, 1998). In our study, GAP-43 mRNA was expressed in almost every pyramidal neuron in each case examined. These observations support the conclusion that there is less GAP-43 mRNA per pyramidal neuron in the DLPFC of patients with schizophrenia.

We did not find decreased GAP-43 mRNA in the hippocampus of patients with schizophrenia. This lack of a reduction was somewhat surprising, because evidence for hippocampal pathology is common in the brain of patients with schizophrenia [reviewed by Weickert and Kleinman (Weickert and Kleinman, 1998)]. Also, damage to the hippocampus in the neonatal rodent can model some aspects of schizophrenia [reviewed by Weinberger and Lipska (Weinberger and Lipska, 1995)]; thus, one might expect to see altered levels of growth-associated markers, such as GAP-43, in the hippocampus of individuals with schizophrenia. Our measurements of GAP-43 mRNA were made in adult humans and may not adequately reflect hippocampal GAP-43 mRNA levels found earlier in development. Additionally, there are, in fact, anatomically specific decreases in GAP-43 mRNA in the hippocampus (Eastwood and Harrison, 1998; Webster *et al.*, 2001) that may not be detected in assays using hippocampal homogenates as we utilized in the RPA analysis in our study.

The decrease of GAP-43 mRNA in the DLPFC of patients with schizophrenia involves pyramidal neurons in at least three different cortical layers. Pyramidal neurons located in cortical layer III can terminate broadly throughout the cortex, in the adjacent cortex and in areas of axon origin through recurrent collaterals (Barbas and Pandya, 1989; Levitt *et al.*, 1993). The reduction of GAP-43 mRNA in pyramidal neurons of layer III in patients with schizophrenia may reflect reduced numbers of cortico-cortical connections. There are reports of a decreased number of postsynaptic spines on layer III pyramidal neurons (Garey *et al.*, 1998; Glantz and Lewis, 2000), reduced levels of the presynaptic vesicular-associated proteins (synaptophysin and SNAP-25) (Glantz and Lewis, 1997; Thompson *et al.*, 1998; Honer *et al.*, 1999; Karson *et al.*, 1999) and increased packing density of neurons (Selemon *et al.*, 1995, 1998) in the DLPFC of patients with schizophrenia. Pyramidal neurons in deep layer III have been reported to be smaller in size in patients with schizophrenia compared with controls (Rajkowska *et al.*, 1998). These results, taken together with our own, support the notion that reductions in the vitality and connectivity of the supragranular pyramidal neuron may be a prominent pathological feature of the DLPFC in patients with schizophrenia. In our study, we also found that subcortically projecting infragranular glutamate pyramidal neurons (layers V and VIa) of patients with schizophrenia had significantly reduced GAP-43 mRNA levels in DLPFC. In primates, neurons in the infragranular layers can project to subcortical sites; DLPFC axons terminating in the caudate nucleus preferentially arise from neurons in layer V and those terminating in the thalamus preferentially arise from neurons in layer VI (Yeterian and Pandya, 1994). Our results suggest that terminals of excitatory corticocaudate and corticothalamic neurons in the brain of patients with schizophrenia may contain less GAP-43 protein and, thus, may be functionally altered. Recent evidence from *in vivo* brain imaging suggests that the frontostriatal circuitry is dysfunctional in the brains of patients with schizophrenia (Manoach *et al.*, 2000).

DLPFC efferents arising in layers III, V and VI can extend to distant cortical regions, including the inferior parietal cortex; the superior, middle and inferior temporal cortices; and the anterior cingulate cortex (Pearlson *et al.*, 1996). A measure of neuronal pathology in the prefrontal cortex of patients with schizophrenia correlates with the activation of the temporal cortex and inferior parietal cortex during working memory tasks (Bertolino *et al.*, 2000). Therefore, it is possible that a decrease in GAP-43 mRNA in cortically projecting neurons of the schizophrenic DLPFC may relate to a decrease in the ability of DLPFC neurons to recruit other cortical areas necessary to perform integrative cognitive tasks (Weinberger, 1993; Goldman-Rakic and Selemon, 1997; Lewis, 1997).

Notes

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