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Evaluation of Neuronal Dysfunction in Schizophrenia before and after Neuroleptic Treatment by ^{1}H MRS

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Abstract: Localized *in vivo* proton magnetic resonance spectroscopy (MRS) was performed to evaluate metabolic alterations in the right and left frontal lobe before and after neuroleptic treatment of schizophrenic patients (n=24) and a group of healthy normal subjects (n=20). Proton metabolic ratios obtained from the 8 cm^3 voxels in the right and left frontal lobe were compared with the clinical assessment of PANSS for each subject. There was no significant difference in the metabolic ratios between the right and the left frontal lobes in either the schizophrenic group or the control group, indicating no laterality. Compared with those of the normal control group, NAA/Cr and (GABA+Glu)/Cr ratios of the schizophrenic patients showed significantly lower (p=0.023) and higher (p=0.005) value, respectively. The (GABA+Glu)/Cr ratio of the schizophrenic patients was generally decreased after neuroleptic treatment, while the NAA/Cr ratio was not changed. Significant correlation between the (GABA+Glu)/Cr ratio and the clinical symptom scores assessed by PANSS was established. The present study supports the "hypofrontality" hypothesis of schizophrenia on the basis of the altered metabolic ratios before and after neuroleptic treatment.

Key Words: proton magnetic resonance spectroscopy, proton metabolites, schizophrenia, frontal lobe

INTRODUCTION

The frontal cortex, which is the most complex and highly developed region in the human brain, would be a logical candidate for studies about the neuropathological mechanism of abnormal thinking, emotion, and behavior in schizophrenia.[1] It has been reported that some schizophrenics have morphological abnormalities of the prefrontal lobe by magnetic resonance imaging (MRI) (Weinberger 1987).[2] In addition, using positron emission tomography (PET) impaired frontal lobe function ("hypofrontalilty") in some schizophrenic

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patients have been reported.[3-4]

Magnetic resonance spectroscopy (MRS) provides noninvasive *in vivo* assessment of brain tissue composition and metabolic processes.[5] Improvements in localization and water-suppressed techniques and the relatively high sensitivity of protons have led to a wide range of application of *in vivo* ^{1}H MRS to the study of human brain tissue.[6] Localized, water suppressed *in vivo* ^{1}H MRS could be used to monitor a number of proton metabolites including putative neurotransmitter amino acids that may be implicated in the pathogenesis of schizophrenia.

In the present study, using *in vivo* ^{1}H MRS the cortex in the frontal lobe was investigated to verify "a hypofrontality hypothesis" in schizophrenia. In addition, the right and left cortex in the frontal lobe was investigated to test "a laterality hypothesis" in schizophrenia.[7] Alterations of metabolite ratios before and after neuroleptic treatment were blindly evaluated and compared with the clinical assessment of PANSS in drug-treated schizophrenia.

METHODS

Subjects

The subjects of this study were 24 inpatients (11 men and 13 women, 17-57 years, mean duration of illness 3.6 years) who were admitted to the Department of Psychiatry, Kangnam St. Mary's Hospital, Catholic University Medical College. They met DSM-III-R criteria[8] for schizophrenia and gave informed consent to our MRS study. Patients with head injuries, neurologic disorder and organic brain syndrome were excluded from this study. Global psychiatric symptomatology and positive and negative symptoms were assessed by the Positive and Negative Syndrome Scale (PANSS)[9] Two psychiatrists evaluated the patients' symptoms on the PANSS and the scores given were averaged to obtain the score for each item. The patients were classified according to DSM-III-R subtypes: disorganized (n=5), paranoid (n=13), undifferentiated (n=6). Ten schizophrenic patients were drug-naive and the remaining fourteen patients who had been previously treated with various neuroleptics, were neuroleptic medication-free for at least 6 months prior to the study.

The control subjects were twenty normal healthy volunteers (10 men and 10 women, 24-35 years) with no known personal or family history of psychiatric disorder.

The handedness inventory revealed that twenty-one of the schizophrenic patients were right-handed and three were mixed handed; sixteen of the control subjects were right-handed and four mixed-handed.

After the initial MRS examinations, the follow-up MRS examinations were performed 8 to 12 weeks after neuroleptic treatment. In the 24 schizophrenic patients, 19 patients (9 males and 10 females, 19-49 years) had successful follow-up examinations. The rest of the

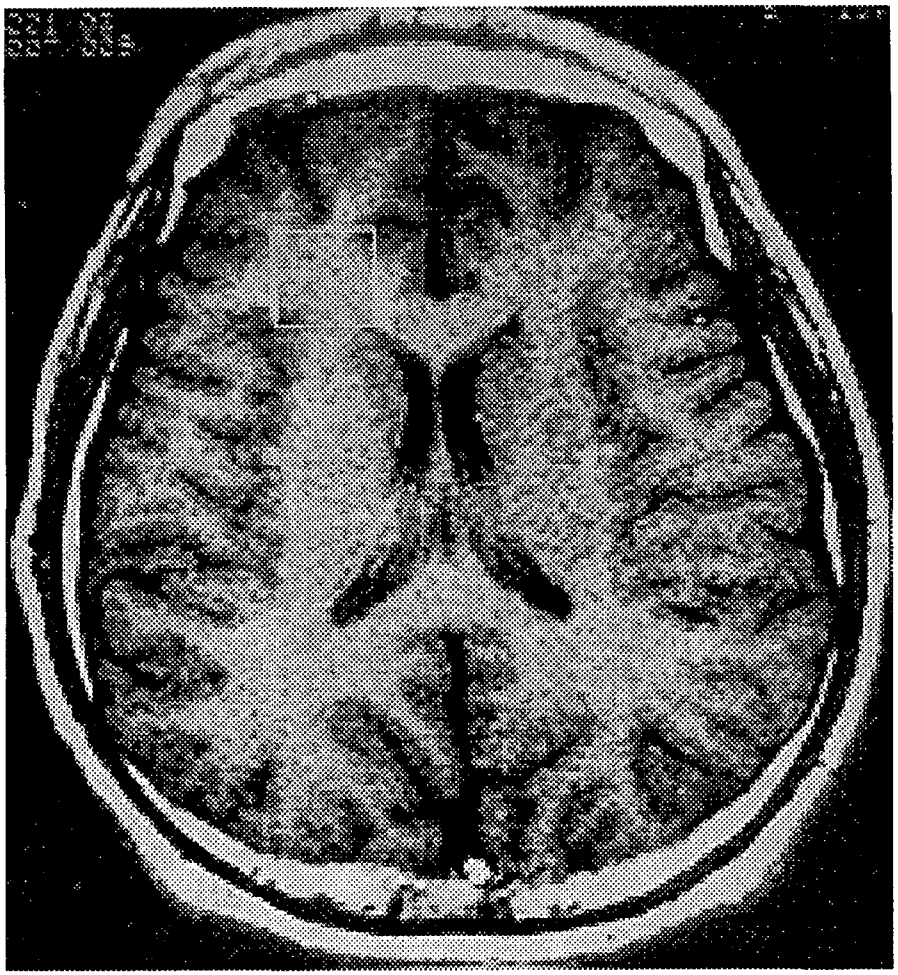

Fig. 1. T1-weighted axial MR image of schizophrenia with the right and left frontal voxels selected for localized *in vivo* ^{1}H MRS.

patients did not undergo or refused repeat MR spectroscopy. The neuroleptic used was haloperidol. The dosages ranged from 5 mg to 25 mg per day.

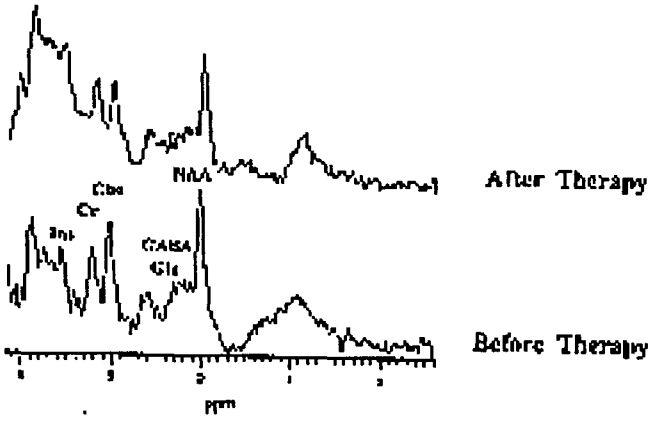

Fig. 2. Typical *in vivo* ^{1}H MR spectra obtained before and after therapy in schizophrenia. Chemical shifts are indicated in parts per million (ppm).

MRS Procedure

^{1}H MRS was performed on a 1.5 T MRI/MRS system (GE Signa Advantage, version 4.8; GE Medical System, Milwaukee, WI) using a stimulated echo acquisition mode (STEAM) pulse sequence.[10-11] A 2x2x2 cm^3 (8ml) voxel in the frontal lobe was selected using the T1-weighted MR images (TE=20 msec; TR=400 msec). *In vivo* ^{1}H MR spectra were obtained from voxels in both the right and the left frontal lobes of schizophrenic patients both before and after neuroleptic treatment (Figure 1). Similarly, *in vivo* ^{1}H MR spectra were obtained from control subjects. Spectral parameters were: TE=20 msec; TR=2000 msec; 128 averages; 2500-Hz spectral width; 2048 data points. The total examination time per case was approximately 40 minutes. All of the *in vivo* ^{1}H MR spectra were acquired with the use of the standard birdcage quadrature head coil (GE Medical Systems, Milwaukee, WI) that produces a uniform radio frequency field of 63.86 MHz. Raw data were transferred to a Sun SPARC station IPC (Sun Microsystems, Mountain View, CA) and processed by the SAGE data analysis package (GE Medical Systems, Milwaukee, WI).

The shimming procedure focused on the water signal was performed to obtain a uniform and homogeneous magnetic field. Typical water line width (full width at half maximum) was 3 to 4 Hz. Special attention was given to locating the water signal frequency to maximize the water suppression. An exponential line broadening of 0.5 Hz was applied. Time domain data were converted to frequency domain by Fourier transformation. Frequency domain spectra were phased by hand, using of frequency-independent phase corrections only. Phased absorption spectra are reported directly without baseline correction or resolution enhancement. All of the ^{1}H MR spectra were plotted and analyzed in a masked fashion in the absorption mode, and fitted to Lorentzian lineshapes. Peak area for each proton metabolite

Table 1. Demographic and Clinical Characteristics of Schizophrenic Patients and Normal Controls

	Mean±SD or Prevalence	
	Controls (n=20)	Schizophrenia (24)
Gender (M/F)	10/10	11/13
Age (yr)	29±3.4	31±6.1
Education level (yrs)	14.7±1.9	13.1±2.5
Duration of illness (yrs)		3.6±3.1
PANSS		
Positive scores		28.6±5.7
Negative scores		23.2±3.6
General psychopathology		45.1±3.4

PANSS = positive and negative syndrome scale

Table 2. Proton Metabolic Ratios of The Normal Control and Schizophrenic Patients.

Metabolite Ratios		Mean values ± SD		ANOVA repeated measure		
		Controls (n=16)	Schizophrenics (n=24)	Source	F	p-value
Ins/Cr	t.	0.90±0.14	0.77±0.16	Group	3.51	NS
	t.	0.80±0.21	0.76±0.15	Side	1.47	NS
				Side by Group	1.16	NS
Cho/Cr	t.	0.86±0.20	0.82±0.13	Group	1.45	NS
	t.	0.74±0.20	0.88±0.20	Side	0.45	NS
				Side by Group	3.01	NS
(GABA+Glu)/Cr	t.	0.50±0.10	0.78±0.22	Group	18.63	0.0001
	t.	0.59±0.13	0.77±0.25	Side	0.55	NS
				Side by Group	0.90	NS
NAA/Cr	t.	1.39±0.23	1.20±0.12	Group	9.05	0.005
	t.	1.36±0.29	1.21±0.16	Side	0.04	NS
				Side by Group	0.26	NS

Cho: choline/phosphocholine, Cr: creatine/phosphocreatine, Ins: Inositol
GABA: gamma aminobutyric acid, Glu: glutamate,
NAA: N-acetylaspartate, L: left, R: right, NS: not significant

was measured using a Marquardt algorithm.[12]

Proton resonance's in the spectra obtained from brain tissues were assigned on the basis of prior assignments.[11] Resonance peak assignments of major ^{1}H MRS observable metabolites were CH_3 of NAA, 2.00 ppm; N-CH_3 of Cr, 3.00 ppm; N-$(CH_3)_3$ of Cho, 3.20 ppm; γ-CH_2 of Glu, 2.35 ppm; γ-CH_2 of GABA, 2.25 ppm; H4 and H6 of Ins, 3.50 ppm. It is very complicated to resolve GABA and Glu at 1.5 T *in vivo,* although the chemical shifts of γ -CH_2 groups of GABA and Glu were assigned as 2.25 and 2.35 ppm,[11] or 2.30 and 2.35 ppm, [13] respectively. Both γ-CH_2 groups of GABA and Glu are a triplet around 2.3 ppm. Since γ-CH_2 groups of GABA and Glu were not convincingly resolved in the region from 2.25-2.35 ppm, they were approximated with two single peaks at 2.25 and 2.35 ppm,[11] respectively. Then, it was achieved to combine GABA and Glu into a single measure GABA+Glu. Only γ-CH_2 groups of GABA and Glu were particularly considered because α- and β-CH_2 groups were severely overlapped with the other major metabolites.[13] To obtain the relative metabolite ratios, Cr was used as a putative reference.[14]

Statistics

Statistical analysis was performed using SAS (statistical analysis of system) 5.0 software. Analysis of variance with repeated measure was used for the major analyses. The dependent variables were relative proton metabolic ratios, group was the between-subjects factor, and side (left versus right) was the within-subjects repeated measures factor. Regression analysis was used to examine the association between proton metabolite ratios and psychiatric symptomatology as assessed by PANSS scores.

RESULTS

There was no significant group difference for demographic findings between the schizophrenic patients and control groups. No anatomical abnormalities or atrophy were found on the MR images of the patients or the controls.

As shown in Table 2 the pretreatment schizophrenic patients had significantly lower ratios of NAA/Cr compared to normal controls (ANOVA, F=9.05, P=0.005). In addition, a significant increase in the (GABA+Glu)/Cr ratio was found in schizophrenics (ANOVA, F=18.63, P=0.0001). However there were no significant differences between proton metabolites in the right and the left frontal lobes in either the schizophrenic group or the control group.

Table 3 shows the relative proton metabolic ratios for both frontal lobes in the schizophrenic patients before and after neuroleptic treatment. The comparison of the ratios of the pre- and post-treatment showed that (GABA+Glu)/Cr ratio was significantly decreased

Table 3. Proton metabolic ratios of before and after treatment in schizophrenic patients:

Metabolite Ratios		Mean values ± SD		ANOVA repeated measure		
		Before Treatment (n=19)	After reatment (n=19)	Source	F	p-value
Ins/Cr	t.	0.74±0.16	0.71±0.19	Group	0.38	NS
	t.	0.77±0.17	0.74±0.23	Side	0.43	NS
				Side by Group	0.00	NS
Cho/Cr	t.	0.83±0.13	0.83±0.13	Group	2.31	NS
	t.	0.90±0.21	0.80±0.17	Side	0.17	NS
				Side by Group	1.61	NS
(GABA+Glu)/Cr	t.	0.78±0.22	0.64±0.17	Group	10.34	0.0027
	t.	0.76±0.26	0.57±0.19	Side	0.83	NS
				Side by Group	0.28	NS
NAA/Cr	t.	1.27±0.17	1.16±0.15	Group	3.90	NS
	t.	1.21±0.17	1.17±0.16	Side	0.66	NS
				Side by Group	1.20	NS

after neuroleptic treatment (ANOVA, F=10.34, P=0.027). However, there were no significant changes in the ratios of NAA/Cr and other proton metabolites before and after neuroleptic treatment. There was no significant correlation between the proton metabolic ratios and the clinical symptom scores assessed by PANSS.

DISCUSSION

The major finding of our present study was that the ratios of NAA/Cr in the right and left frontal lobes in schizophrenics were significantly lower compared to the control group. Our results also showed that there were no significant differences in ratios of NAA/Cr before and after neuroleptic treatment. According to the report that the brain concentration of Cr is fairly constant in various metabolic conditions,[15] we think that the decreased ratios of NAA/Cr in schizophrenic patients must be caused by an NAA reduction.

However, the exact significance of our result is not yet clear. The biochemical functions of proton metabolites in brain tissues are not yet fully understood. Since NAA, the largest signal in a healthy brain, is assumed to be confined to neurons and believed to be a neuronal marker,[16] a reduced level of NAA may indicate a form of neuronal dysfunction or loss of neurons in the frontal lobe in patients with schizophrenia. Thus, these findings appear

to support the hypothesis that NAA reductions may be related to hypofrontality. The observation that reduced NAA levels did not change following neuroleptic treatment may also indicate trait-dependent abnormalities in schizophrenia.

Although only the neuroleptic-naive or drug-free (for at least 6 months) schizophrenic patients were chosen, to exclude any psychotropic medication effects, our results should be interpreted with caution because the residual effects of neuroleptic drugs on the proton spectra have not been determined. recent report in examining neuroleptic-naive schizophrenic patients' frontal cortexes[17] found no difference in NAA levels.

In addition, the localization of VOI must be considered before reaching conclusions. Since the VOI included both gray and white matter, their relative admixture may account for relative values of NAA and Cr. As NAA appears to be more concentrated in the gray matter than in the white matter,[18] a reduced proportion of gray matter in the VOI may lead to a decrease in the NAA/Cr ratios.

The second major finding in this study was the increase in (GABA+Glu) in the right and left frontal lobes of the schizophrenics that was significantly decreased following neuroleptic treatment. Interestingly the decrease in (GABA+Glu) had no significant correlation with either clinical improvement or medication dosage following neuroleptic treatment.

As GABA and Glu were not resolved convincingly at 1.5 T (relatively low field) in vivo, it was technically difficult to identify the γ-CH_2 groups of GABA and Glu in the structures noted between 2.25 to 2.35 ppm. As the separation of only 0.1 ppm further complicated quantity of peak areas, it was more conservative to combine GABA and Glu into a single measure. However, the unique identification of GABA can be done using homonuclear spectral editing techniques like that used by Rothman *et al.*[19] Although approximation of a single measure of (GABA + Glu) is more conservative in the quantification of severely overlapping peaks, it has some distinct drawbacks. First, because a single measure can't reveal the specific identification of GABA or Glu in the metabolic alterations, it is impossible to identify which compound would be definitely altered following neuroleptic treatment. Second, in addition to GABA and Glu, a single measure may contain other amino acids such as glutamine as the chemical shift of γ-CH_2 groups of glutamine is 2.45 ppm.[11] Third, two single peaks at 2.25 and 2.35 ppm could drift because the chemical shift difference 0.1 to 0.2 ppm is quite sensitive to temperature variations. Under unstable temperatures, spectra might not provide reliable quantitative data.

The increase of the (GABA+Glu) level in schizophrenic patients may indicate abnormal neuronal function in neurotransmitters. The (GABA+Glu) levels of drug-treated schizophrenics generally were changed toward those of control subjects, indicating metabolic improvement. The reduction of GABA and Glu in drug-treated schizophrenia may be implicated in the balancing glutamate-mediated neuronal excitation and the recovery from neneuropsychiatric

disorders, and the returning to normal of metabolic processes in the mechanism for subcortical dopaminergic hyperactivity with prefrontal deafferentation.[20]

In conclusion, we found evidence of the alteration of proton metabolites of the frontal lobe that have been hypothesized to be involved in hypofrontalilty of schizophrenia. Further studies in other locations of the brain with a larger sample size would be necessary to expand our understanding of altered proton metabolites in schizophrenia.

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REFERENCES

1. Andreasen NC, Rezai K, Alliger R, Swayze II VW, Flaum M, Kirchner P, Cohen G, and O'Leary S, Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia, *Arch Gen Psychiatry* , **49**, 943-958 (1992).
2. Andreasen NC, Nasrallah HA, Dunn V, Olson SC, Grove WM, Ehrhardt JC, Coffman JA, and Crossett JHW, Structural abnormalities in the frontal system in schizophrenia - a magnetic resonance imaging study, *Arch Gen Psychiatry*, **43**, 136-144 (1986).
3. Buchsbaum MS, Ingvar DH, Kessler R, Waters RN, Cappeletti J, Van Kammen DP, King AC, Johnson JL, Manning RG, Flyn RW, Mnn LS, Bunney WE, Jr, and Sokoloff L, Cerebral glucography with positron emission tomography, *Arch Gen Psychiatry*, **39**, 251-259 (1982).
4. Buchsbaum MS, Nuechterlein KH, Haier RJ, Wu J, Sicotte N, Hazlett E, Asarnow R, Potkin S, and Guich S, Glucose metabolic rate in normals and schizophrenics during the continuous performance test assessed by positron emission tomography, *Br J Psychiatry* , **156**, 216-227 (1990).
5. Lock T, Abou-Saleh MT, and Edwards RHT, Psychiatry and the new magnetic resonance era. *Br J Psychiatry*, **157**, 38-55 (1990).
6. Bottomley PA, Human *in vivo* NMR spectroscopy in diagnostic medicine - clinical tool or research probe, *Radiology*, **170**, 1-15 (1989).
7. Gur RE, Resnick SM, and Gur RC, Laterality and frontality of cerebral blood flow and metabolism in schizophrenia - relationship to symptom specificity, *Psychiatry Res.*, **27**, 325-334 (1989).
8. American Psychiatric Association, DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders. 3rd ed., revised. Washington, DC: American Psychiatric Press, 1987.
9. Kay SR, Opler LA, and Lindenmayer JP, Reliability and validity of the positive and negative syndrome scale for schizophrenics, *Psychiatry Res.*, **23**, 99-110 (1988).

10. Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, and Sauter R, Localized high-resolution proton NMR spectroscopy using stimulated echoes - Initial applications to human brain in vivo, *Magn Reson Med.*, **9**, 79-93 (1989).
11. GE, Marguardt fitting. In: *SA/GE spectroscopy analysis workstation user's manual and software installation instructions*, revision 1, 43-46, GE. Milwaukee, Wisconsin, USA, GE Medical system, 1990.
12. Kreis R, Farrow N, and Ross B, Localized ^{1}H NMR spectroscopy in patients with chronic hepatic encephalopathy - analysis of change in cerebral glutamine, choline, and inositols, *NMR in Biomed.*, **4**,109-116 (1991).
13. Petroff OAC, Spencer DD, Alger JR, and Prichard JW, High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy, *Neurology*, **39**, 1197-1202 (1989).
14. Ross B, Kreis R, and Ernst T, Clinical tools for the 90s - magnetic resonance spectroscopy and metabolic imaging, *Eur J Radiology*, **14**, 128-140 (1992).
15. Petroff OAC, Ogino T, and Alger JR, High-resolution proton magnetic resonance spectroscopy of rabbit brain - regional metabolic levels and postmortem changes, *J Neruochem.*, **51**, 163-171 (1988).
16. Breiter SN, Barker PB, Mathews VP, Arroyo SS, and Bryan RN, In: Book of Abstracts: *Society of Magnetic Resonance in Medicine*, Vol.1, Berkeley Calif., 1992, *Society of Magnetic Resonance in Medicine*, 644 (1992).
17. Stanly JA, Drost DJ, Williamson PC, Carr T, Rylett J, and Merskey H, The study of schizophrenia via vivo 31P and 1H MRS, *Schizophr Res.*, **9**, 210 (1993).
18. Koller KJ, Zaczek R, and Coyle JT, N-acetyl-aspartyl-glutamate - regional levels in rat brain and the effects of brain lesions as determined by a new HPLC method, *J Neurochem.*, **43**, 1136-1142 (1984).
19. Rothman DL, Petroff OAC, Behar KL, and Mattson RH, Localized ^{1}H MRS measurements of GABA in human brain, In: book of abstracts: *Society of Magnetic Resonance in Medicine*, Berkeley. CA., 1993.
20. Tasman A, and Goldfinger SM, *American Psychiatric Press review of psychiatry*, Vol. 10, Washington DC: American Psychiatric Press, 1991,: Weinberger DR, Implications of normal brain development for the pathogenesis of schizophrenia, *Arch Gen Psychiatry*, **44**, 660-669 (1987).