

Metabolite Alterations in Basal Ganglia Associated with Psychiatric Symptoms of Abstinent Toluene Users: A Proton MRS Study

Kiyokazu Takebayashi¹, Yoshimoto Sekine¹, Nori Takei^{*1,2,3}, Yoshio Minabe¹, Haruo Isoda⁴, Hiroyasu Takeda⁴, Katsuhiko Nishimura¹, Kazuhiko Nakamura¹, Katsuaki Suzuki¹, Yasuhide Iwata¹, Harumi Sakahara⁴ and Norio Mori¹

¹Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; ²Stanley Foundation Research Center in Japan, Hamamatsu, Shizuoka, Japan; ³Department of Psychological Medicine, Institute of Psychiatry, London, UK; ⁴Department of Radiology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan

Long-term toluene abuse causes a variety of psychiatric symptoms. However, little is known about abnormalities at the neurochemical level in the living human brain after long-term exposure to toluene. To detect neurochemical changes in the basal ganglia of subjects with a history of long-term toluene use, proton magnetic resonance spectroscopy (¹H MRS) was performed in 12 abstinent toluene users and 13 healthy comparisons with no history of drug abuse. *N*-acetylaspartate (NAA), creatine plus phosphocreatine (Cr + PCr), choline-containing compounds (Cho), and myo-inositol (MI) levels were measured in the left and right basal ganglia. The Cho/Cr + PCr ratio, a marker of membrane metabolism, was significantly increased in the basal ganglia of toluene users in comparison to that of the control subjects. Furthermore, the increase in the Cho/Cr + PCr ratio was significantly correlated with the severity of residual psychiatric symptoms. These findings suggest that long-term toluene use causes membrane disturbance in the basal ganglia, which is associated with residual psychiatric symptoms that persist even after long-term abstinence from toluene use.

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INTRODUCTION

Organic solvent abuse is a worldwide problem, particularly among adolescents (Flanagan and Ives, 1994; Neumark *et al*, 1998). The easy accessibility to organic solvents makes them among the first drugs tried by teenagers. Consequently, teenagers are liable to develop an addiction to organic solvents, and such use can serve as a 'gateway' to the ingestion of other illicit drugs (Ives, 2000). Toluene is one of the major components of many organic solvents and is highly lipid-soluble (Morton, 1987). Toluene rapidly permeates lipid-rich regions of the body such as the brain, and its recreational intake can cause chronic and persistent

psychiatric symptoms, including hallucinations and alterations in personality, even after cessation of toluene use (Hormes *et al*, 1986; Morton, 1987). However, the mechanisms responsible for these residual psychiatric symptoms following chronic toluene use remain unknown.

In experimental animal studies, exposure to toluene has been shown to produce changes in lipid membrane fluidity and membrane function (Kyrklund *et al*, 1987; LeBel and Schatz, 1990; von Euler *et al*, 1990). Furthermore, long-term exposure to toluene induces a persistent dopaminergic dysfunction in the rat basal ganglia, and this toluene-induced dopaminergic dysfunction has been related to the behavioral changes and the persistent cognitive deficits in rats (Cintra *et al*, 1999; Hillefors-Berglund *et al*, 1995; von Euler *et al*, 1993, 2000).

In human MR studies of toluene users, T2-weighted magnetic resonance images revealed global atrophy in the cerebrum, cerebellum, and brainstem (Rosenberg *et al*, 1988). Loss of differentiation between gray and white matter, increased periventricular white matter signal intensity (Aydin *et al*, 2002; Filley *et al*, 1990; Rosenberg *et al*, 1988, 2002), and hypointensity in the thalamus and

*Correspondence: Dr N Takei, Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192 Shizuoka, Japan, Tel: +81 53 435 2295, Fax: +81 53 435 3621,

E-mail address: ntakei@hama-med.ac.jp

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basal ganglia (Rosenberg *et al*, 2002; Unger *et al*, 1994) have also been found in toluene users. Furthermore, white matter changes have been reported to be associated with neurocognitive deficits in long-term toluene users (Filley *et al*, 1990). However, little is known about the effects of long-term toluene use on the human brain at the neurochemical level.

Proton magnetic resonance spectroscopy (^1H MRS) offers an opportunity to evaluate *in vivo* neurochemical changes that might occur in toluene users. In this study, we investigated the potential of ^1H MRS scanning for revealing neurochemical changes in the chemistry of the basal ganglia of abstinent toluene users; we also considered whether or not such changes, if found, could possibly be related to the clinical characteristics observed in abstinent toluene users.

METHODS

Subjects

A total of 12 toluene users and 13 healthy comparison subjects who had never used toluene were recruited for our comprehensive research project on substance-related psychiatric disorders. Candidates were excluded if they were seropositive for human immunodeficiency virus type-1 (HIV-1), if they had a history of head trauma with loss of consciousness, or if they had brain morphological abnormalities (eg atrophy or tumor, etc) on MR images. All participants showed no neurological abnormalities and had no history of stupor or coma, which may be observed during intoxication after the use of high doses of toluene. All subjects were screened using the Structured Clinical Interview for DSM-IV (SCID) (First *et al*, 1996). More specifically, detailed information on toluene use and the history of psychiatric symptoms was retrospectively obtained from the toluene users; SCID-based interviews with the users and their family members were conducted, and patient medical records were considered as well. None of the toluene users had used any other drugs (ie they were mono-drug users) and the subjects had had no history of DSM-IV mental disorders before the use of toluene. The comparison subjects had no experience of the use of any illicit drugs, and had no history of mental disorders, including substance-related disorders, according to the DSM-IV. Both groups reported similar occasional smoking and drinking habits, and none of the subjects matched the DSM-IV criteria for either nicotine- or the alcohol-related disorders. The toluene users were recruited from the Hamamatsu University Hospital, and from three associated hospitals (Hattori Mental Hospital, Shimada Municipal Hospital, and Hamamatsu Neurological Hospital).

The duration of toluene use was defined as the time that had intervened between the initial and the last use of toluene. In cases involving intervals of abstinence exceeding 1 month during the duration of toluene use as defined, these intervals were subtracted from the total length of toluene use. The duration of abstinence from toluene use was defined as the time between the day of the last use of toluene and the day of the ^1H MRS examination. Cumulative lifetime exposure to toluene was estimated in milliliters. Among the 12 toluene users, five had been treated with neuroleptics, while the remaining seven toluene users had

not previously taken neuroleptics prior to the ^1H MRS examination. Current and total lifetime neuroleptic doses in the five toluene users were quantified in chlorpromazine-equivalent dosages (American Psychiatric Association, 1997). In order to establish recent abstinence from toluene use, we regularly tested the subjects for urinary hippuric acid, a biomarker of toluene use (Raikhlin-Eisenkraft *et al*, 2001), using high-performance liquid chromatography, which was carried out according to the standard diagnostic methods. In addition, to verify abstinence from other illicit drugs, we used a rapid immunoassay for the qualitative detection of the metabolites of the following eight classes of drugs: amphetamines (including (\pm)3,4-methylenedioxy-methamphetamine and methamphetamine), barbiturates, benzodiazepines, cocaine, methadone, opiates, tetrahydrocannabinol, and tricyclic antidepressants (Triage 8, Biosite Diagnostics, San Diego, USA). These assessments were also performed on the same day as the ^1H MRS examination. Psychiatric symptoms in the toluene users were evaluated using the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) on the day of the ^1H MRS study. The assessment was conducted by a trained research psychiatrist blind to the ^1H MRS data. After providing the subjects with a complete description of the study, written informed consent was obtained from all participants before entry into the study, in compliance with the procedures of the Ethics Committee of the Hamamatsu University School of Medicine.

Image Acquisition

MRS was performed at the Hamamatsu University Hospital, using a 1.5-T whole-body MR scanner (Signa Horizon; General Electric Medical Systems, Milwaukee, WI, USA) equipped with self-shielded gradient coils. A quadrature, MR bird-cage coil (trans/receive) was used throughout the study. The localized MR spectra were recorded using the point-resolved spatially localized spectroscopy (PRESS) sequence from the bilateral basal ganglia. The RF transmitter frequency was centered at the water resonance frequency (63.88 MHz). A 90° flip angle was determined by varying the RF amplifier gain until a maximal signal consistent with a good slice profile was obtained. The B_0 shim on the 27 ml cubic excited volume was optimized using linear shims to a full-width at half-maximum (FWHM) of <6.4 Hz. Voxel size was 8 cm^3 ($2 \times 2 \times 2\text{ cm}^3$). To establish the voxel of interest (VOI) on an MR image, scout views in the proton density images were obtained vertically in the Z-axis (Figure 1). Proton density images were acquired because gray matter contrasts well with white matter in this type of imaging study (Sekine *et al*, 2002).

After the location of the VOI had been verified by localized MR imaging, the magnetic field in the VOI was optimized by means of a water signal to establish a linewidth of <3 Hz. The acquisition parameters were as follows: repetition time (TR), 1500 ms; echo time (TE), 40 ms; and acquisition time, 3 min 42 s. Peaks of N-acetylaspartate (NAA) (2.0 ppm), creatine plus phosphocreatine (Cr + PCr) (3.0 ppm), choline-containing compounds (Cho) (3.2 ppm), and myo-inositol (MI) (3.6 ppm) were obtained. Data processing were performed using a PROBE/SV-Quant tool (General Electric Medical Systems,



Figure 1 Axial slice proton density MRI showing the typical location of the MRS voxel. The right side of the figure represents the left side of the brain.

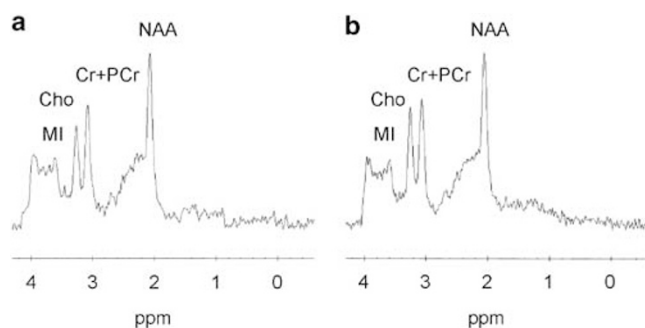


Figure 2 Proton MRS spectra for the right basal ganglia region of (a) a comparison subject and (b) a toluene user. MI, myo-inositol; Cho, choline-containing compounds; Cr + PCr, creatine plus phosphocreatine; NAA, N-acetylaspartate.

Milwaukee, WI, USA), which employs a standard nonlinear least-squares algorithm for fitting the observed data. The metabolite ratios were calculated from the fitted peak integrals in the resulting spectra using a method of automatic metabolite peak measurement (Kreis *et al*, 1991; Webb *et al*, 1994). Representative spectra for the right basal ganglia region of a comparison subject and a toluene user are shown in Figure 2. The ratios of NAA/Cr + PCr, MI/Cr + PCr, Cho/Cr + PCr, and Cho/NAA in the toluene users were compared with those of the comparison subjects.

It should be noted that we were unable to completely exclude potential cerebrospinal fluid (CSF) contamination. All measurements were determined by reference to a standard neuroanatomic atlas (Daniels *et al*, 1987), and great care was taken to minimize CSF contamination. However, measurement of tissue water signal from the voxel was too variable owing to a tissue/ventricle artifact to be used to calculate absolute concentrations of metabolites. Accordingly, only the metabolite ratios are reported here.

Statistical Analysis

Student's *t*-test was used for the comparison of the means of the metabolite ratios between toluene users and comparison subjects. The relationships between the metabolite ratios and clinical parameters (ie the duration of toluene use, the duration of abstinence, the lifetime amount of toluene use, the current neuroleptic dose, the lifetime neuroleptic dose, and the BPRS score) were evaluated using Pearson's correlation coefficient. We measured the metabolite ratios in the basal ganglia on both the left and right sides of each subject. The values measured in each of the hemispheres were not naturally independent within the same individual, and separate analyses of the ratios, as measured on each side, would have increased the risk of Type I error due to multiple testing. To avoid such errors, we used repeated measures analysis of variance (ANOVA), in which the repeated measures were the left and right metabolite ratios. In all of the statistical analyses, an alpha level of 0.05 was considered to be significant.

RESULTS

The demographic and clinical characteristics of the toluene users and the comparison subjects are shown in Tables 1

Table 1 Demographic Characteristics of Toluene Users and Comparison Subjects

	Toluene users (n = 12)	Comparison subjects (n = 13)	Significance
Age (years)	29.6 (6.5)	25.9 (6.3)	NS ^a
Gender (male/female)	9/3	10/3	NS ^b
Duration of use (years)	7.8 (5.1)	—	—
Duration of abstinence (years)	4.9 (4.9)	—	—
Total lifetime exposure of toluene (liters)	2 298 (4 900)	—	—
BPRS total score	16.1 (9.6)	—	—

Data are presented as mean (SD). BPRS, the Brief Psychiatric Rating Scale; NS, nonsignificant.

^aNS, by *t*-test.

^bNS, by Fisher's exact test.

Table 2 Clinical Characteristics of Toluene Users

Subject	Age (years)	Sex	Age of first toluene use (years)	Duration of toluene use (years)	Duration of abstinence (years)	Lifetime exposure toluene (ml)	Neuroleptic dose at scanning ^a (mg/day)	Lifetime neuroleptic dose ^a (mg)	BPRS score	Symptoms at time of MRS
1	21	m	15	3	3.5	17 460 000	0	0	5	Auditory hallucinations
2	23	m	15	5	3.0	2 737 500	0	0	14	Persecutory delusions Unusual thought content
3	32	m	23	11	0.3	231 000	0	0	25	Persecutory delusions Emotional apathy
4	36	m	18	12	10.0	9 125 000	175	1 420 500	24	Emotional apathy
5	32	m	14	6	1.0	3 355 500	400	9 000	19	Somatic delusions Emotional apathy
6	18	f	15	1	3.0	14 400	0	0	11	Anxiety Excitement
7	30	f	16	7	7.0	1 404 000	0	0	3	Anxiety
8	41	m	13	17	3.0	602 357	1 827	9 072 000	33	Auditory hallucinations Emotional apathy
9	28	m	15	13	0.1	787 200	100	237 000	27	Somatic delusions Auditory hallucinations Emotional apathy
10	34	f	15	4	14.0	7821	0	0	16	Anxiety Depressive mood
11	32	m	17	3	13.0	16 965	400	677 775	10	Auditory hallucinations Excitement
12	28	m	14	13	1.0	47 400	0	0	6	Emotional apathy

^aNeuroleptic dosages are in chlorpromazine equivalents.

Table 3 Metabolite Ratios in the Left and Right Basal Ganglia of Toluene Users ($n = 12$) and Comparison Subjects ($n = 13$)

	NAA/Cr+PCr	MI/Cr+PCr	Cho/Cr+PCr ^a	Cho/NAA ^b
<i>Toluene users</i>				
Left basal ganglia	1.41 (0.13)	0.41 (0.08)	0.80 (0.08)	0.57 (0.07)
Right basal ganglia	1.38 (0.10)	0.45 (0.06)	0.82 (0.09)	0.60 (0.09)
<i>Comparison subjects</i>				
Left basal ganglia	1.35 (0.18)	0.47 (0.18)	0.72 (0.07)	0.53 (0.05)
Right basal ganglia	1.44 (0.17)	0.42 (0.08)	0.74 (0.07)	0.52 (0.05)

Data are presented as mean (SD). NAA, N-acetylaspartate; Cr+PCr, creatine plus phosphocreatine; MI, myo-inositol; Cho, choline-containing compounds.

^a $p = 0.006$.

^b $p = 0.02$ for toluene users vs comparison subjects, by repeated measures ANOVA in which the repeated measures were the left and right metabolite ratios.

and 2. No significant difference was observed as regards the distribution of age or sex between the toluene users and the comparison subjects. The mean age of the first inhalation in toluene users was 15.8 years (range: 13–23). While one toluene user had a first-degree relative with alcoholism, none of the other users had any first- or second-degree relatives with any psychiatric disorders. The 12 toluene users, with or without neuroleptic treatment, showed similar clinical symptoms, such as auditory hallucinations, persecutory delusions, and emotional apathy. However,

they exhibited no noticeable diminished volition, incongruity of affect, stereotypy, mannerism, or loosening of association, and their interest in social activities and interpersonal relationship were fairly maintained. Among the 12 toluene users, five had been treated with neuroleptics, including chlorpromazine, levomepromazine, haloperidol, risperidone, and quetiapine. The neuroleptics successfully improved psychotic symptoms characterized by delusions or hallucinations, but were less effective for emotional apathy.

The metabolite ratios for the left and right basal ganglia are shown in Table 3. Repeated measures ANOVA revealed no significant interaction of group and laterality (left vs right) in the metabolite ratios measured, which suggested that any differences observed between the ratios of the two groups were similar in the left and right basal ganglia. There was no significant difference in the NAA/Cr + PCr ratio between the two groups ($F=0.001$, $df=1,23$, $p=0.98$). When the toluene users were divided into two groups, that is, medicated ($n=5$) and nonmedicated ($n=7$), no significant difference between either of these groups and the comparison subjects was found ($F=0.06$, $df=1,16$, $p=0.81$ for the medicated group; $F=0.06$, $df=1,18$, $p=0.81$ for the nonmedicated group). There was also no significant difference in the MI/Cr + PCr ratio between the toluene users and the comparison subjects ($F=0.09$, $df=1,23$, $p=0.77$). The difference between the medicated patients and comparison subjects was not significant, nor was that between the nonmedicated patients and comparison subjects ($F=0.001$, $df=1,16$, $p=0.98$ for the medicated group; $F=0.57$, $df=1,18$, $p=0.11$ for the nonmedicated group). However, the toluene users had significantly higher ratios of Cho/Cr + PCr than did the comparison subjects ($F=9.10$, $df=1,23$, $p=0.006$). A similar significance was also noted in the five medicated ($F=10.19$, $df=1,16$, $p=0.006$) and seven nonmedicated ($F=4.50$, $df=1,18$, $p=0.05$) toluene users as compared with the comparison subjects. The ratio of Cho/NAA was significantly higher in the toluene users compared with that of the comparison subjects ($F=6.15$, $df=1,23$, $p=0.02$). The difference in the ratio between nonmedicated toluene users and comparison subjects was of marginal significance ($F=4.01$, $df=1,18$, $p=0.06$), but the difference between medicated toluene users and the comparison subjects was found to be significant ($F=6.24$, $df=1,16$, $p=0.02$).

There was a significant positive correlation between the Cho/Cr + PCr ratio and the BPRS score on the left side of the basal ganglia ($r=0.57$, $df=11$, $p=0.05$) (Figure 3), whereas such a correlation was not found on the right side ($r=0.18$, $df=11$, $p=0.58$). None of the other clinical variables, that is, the duration of toluene use (left: $r=0.09$, $df=11$, $p=0.79$; right: $r=0.05$, $df=11$, $p=0.87$), the duration of abstinence (left: $r=0.25$, $df=11$, $p=0.44$; right: $r=0.18$, $df=11$, $p=0.57$), the total lifetime exposure to

toluene (left: $r=0.42$, $df=11$, $p=0.18$; right: $r=0.24$, $df=11$, $p=0.46$), current neuroleptic dose (left: $r=0.05$, $df=11$, $p=0.87$; right: $r=0.09$, $df=11$, $p=0.78$), or lifetime neuroleptic dose (left: $r=0.006$, $df=11$, $p=0.99$; right: $r=0.26$, $df=11$, $p=0.42$), were correlated with the Cho/Cr + PCr ratio on either side of the basal ganglia. Due to the possibility that the psychiatric symptoms were ameliorated by medication at the time of assessment, we computed the partial correlation coefficient with adjustment for the current neuroleptic dose. As anticipated, a slightly higher correlation emerged between the Cho/Cr + PCr ratio in the left basal ganglia and the BPRS score ($r=0.65$, $df=9$, $p=0.03$), whereas the correlation between Cho/Cr + PCr ratio in the right basal ganglia and the total BPRS score remained nonsignificant ($r=0.27$, $df=9$, $p=0.42$).

DISCUSSION

The present study revealed that the ratio of Cho/Cr + PCr in the bilateral basal ganglia of toluene users was significantly higher than the corresponding value in the comparison subjects. Although the possible change in the Cr + PCr level, as reference, might have modified or obscured actual alterations in the primary metabolites, the Cho/NAA ratio, as well as the Cho/Cr + PCr ratio, was significantly higher in the basal ganglia of the toluene users compared with that of the comparison subjects. Therefore, it follows that changes in the Cho/Cr + PCr ratio may arise from an elevation of Cho in the basal ganglia of toluene users. To our knowledge, this is the first examination of the effects of toluene on the MRS measures of brain chemicals. The Cho/Cr + PCr ratio may be a nonspecific marker of toluene-induced biochemical changes in the basal ganglia in long-term toluene users. However, since five of the 12 toluene users examined received neuroleptic medication, the effect of medication on the metabolite ratio should be considered. Indeed, several ^1H MRS studies of medicated patients with schizophrenia have shown an increased Cho/Cr + PCr ratio in the caudate nucleus (Ando *et al*, 2002; Bustillo *et al*, 2001), although other studies have not (Heimberg *et al*, 1998; Ohara *et al*, 2000). Consequently, at this point, the effect of neuroleptic medication on the metabolite values of brain Cho/Cr + PCr remains controversial. In this study, a significant increase in the Cho/Cr + PCr ratio was observed, in the seven toluene users who had not been medicated. However, the level of significance demonstrated in the seven nonmedicated users was of borderline significance ($p=0.05$), whereas the result was highly significant in the five medicated toluene users ($p=0.006$). Therefore, the use of neuroleptic medication may have exerted some effect on the elevation of the Cho/Cr + PCr ratio in the participants of this study.

The peak of choline-containing compounds on ^1H MRS reflects the concentrations of free choline, phosphocholine, and glycerophosphorylcholine (Miller *et al*, 1996; Stanley *et al*, 2000). The compounds responsible for the choline peak are associated with membrane phospholipids, and have been reported to be increased in a variety of human pathologies that are characterized by increased membrane phospholipid turnover such as demyelinating processes and gliosis; examples include multiple sclerosis (Tartaglia *et al*,

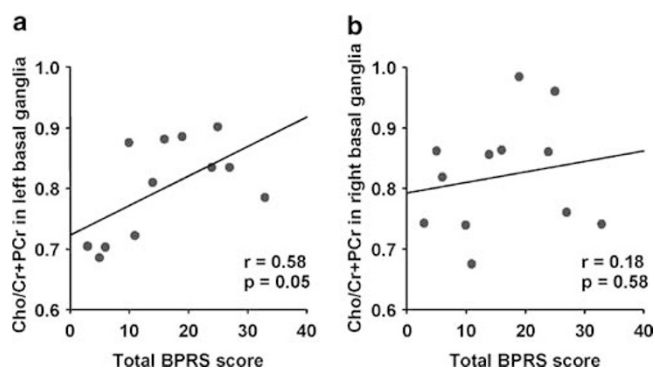


Figure 3 Correlations between clinical variables and values of the Cho/Cr + PCr ratio in the (a) left and (b) right basal ganglia of toluene users ($n=12$). A significant correlation was found only in the left basal ganglia using Pearson's correlation coefficient.

2002), HIV-related brain disease (Chang *et al*, 1999), dementia (Pfefferbaum *et al*, 1999), and epilepsy (Lundbom *et al*, 2001). In animal studies, Gerasimov *et al* (2002) have recently demonstrated using PET that rapid uptake and clearance of radioactivity of [¹¹C]toluene into specific regions of the brain take place, especially in the striatum, and this effect is thought to be the high lipid solubility of this agent. In addition, both acute and long-term exposure of rats to toluene have been shown to alter membrane composition and membrane fluidity (Edelfors and Ravn-Jonsen, 1989; Kyrklund *et al*, 1987; Lebel and Schatz, 1990; von Euler *et al*, 1990). Two post-mortem studies of the brains of toluene users have reported diffuse demyelination in the subcortical white matter, in addition to the gliosis of white and/or gray matter (Escobar and Aruffo, 1980; Rosenberg *et al*, 1988). In human MRI studies, T2-weighted images of toluene abusers showed hypointensity in the basal ganglia and thalamus (Aydin *et al*, 2002; Filley *et al*, 1990; Rosenberg *et al*, 1988; Unger *et al*, 1994), and this type of hypointensity has been suggested to be associated with changes in myelin metabolism (Aydin *et al*, 2002; Rosenberg *et al*, 1988). In addition, demyelinating lesions are thought to contribute to the increase in the Cho/Cr ratio in a patient with multiple sclerosis (Arnold *et al*, 1992). Taken together, the elevated Cho/Cr + PCr ratio in the basal ganglia observed in abstinent toluene users in this study may have been reflective of a long-lasting abnormality in membrane phospholipid metabolism, in particular, demyelination.

The elevation of Cho/Cr + PCr ratios in the left, but not in the right, basal ganglia was significantly correlated with the severity of psychiatric symptoms as measured by the BPRS. The reason for this lateralized dysfunction of the brain in toluene users is not known. However, it is generally accepted that left lateralized dysfunction or structural changes can be observed in neuropsychiatric disorders such as schizophrenia (Hietala *et al*, 1995; Petty, 1999; Reynolds, 1983). In this study, an elevation in the Cho/Cr + PCr ratio was noted among those who had stopped using toluene for 4.9 years on average. Therefore, the abnormalities in membrane phospholipid metabolism observed in these toluene users may be persistent and may participate in the pathogenesis of protracted psychiatric symptoms. Although no prior studies have examined the relationship between psychiatric symptoms and neurochemical alterations in toluene users, there is some evidence linking membrane abnormalities with psychiatric symptoms in patients with schizophrenia (Fenton *et al*, 2000; Fukuzako *et al*, 1996; Ross *et al*, 1997).

In this study, no significant difference was found in NAA/Cr + PCr ratios between the toluene users and the comparison subjects. This finding was not altered when the administration of neuroleptic medication was taken into account. In addition, since NAA has been widely used as a marker of neuronal density (Miller, 1991; Simmons *et al*, 1991; Tsai and Coyle, 1995), our results suggest that toluene abusers may be spared from neuronal loss in the basal ganglia. Indeed, this view is in line with the finding that there was no difference in the MI/Cr + PCr ratio between toluene users and comparison subjects in this study. Since MI is considered to be a marker of glial response (Brand *et al*, 1993), a finding of no change in the MI/Cr + PCr ratio

may represent the absence of neuronal damage in toluene users. Histological studies of rats chronically exposed to toluene have shown no significant neuronal loss in the neocortex or cerebellum (Korbo *et al*, 1993; Ladefoged *et al*, 1991), although one study has shown a reduction in the density of neurons in the hippocampus of toluene-exposed rats (Korbo *et al*, 1996). Furthermore, an enzyme immunoassay study using γ -enolase and calbindin-D28k as neural cell markers showed no quantitative changes in the cerebrum of rats after exposure to toluene (Huang *et al*, 1992). In post-mortem case reports about two toluene users who abused toluene for 12 and 20 years, respectively, one patient was reported to have neuronal loss in the cerebrum, basal ganglia, and cerebellum (Escobar and Aruffo, 1980), while in the other case, no neuronal loss was detected in the cerebrum or cerebellum (Rosenberg *et al*, 1988). Generally, neural cells appear less likely to be compromised by exposure to toluene, although very high doses of toluene can damage neural cells (Hansson *et al*, 1988). However, vulnerability to toluene exposure may vary in different regions of the brain. Therefore, the result showing no change in the NAA/Cr + PCr ratio in our study does not exclude the possibility of neuronal damage occurring in brain regions other than the basal ganglia.

A worldwide comprehensive survey has shown that the majority of toluene users start inhaling toluene when they are teenagers (Kozel *et al*, 1995; Neumark *et al*, 1998). In accordance with that survey, all but one of our toluene users (with the exception starting toluene inhalation at the age of 23 years) began to take toluene while still a teenager, that is, under 18 years of age. Furthermore, the mean age of initiation of toluene use in this study (about 16 years) was far earlier than the mean age of onset of schizophrenia, which has been shown to be in the early 20s (Bromet *et al*, 1995). Although we cannot rule out the possibility that the use of toluene precipitates schizophrenia in vulnerable individuals, as pointed earlier, none of the toluene users had a family history of major psychosis. However, the toluene users in the present study had psychotic symptoms akin to those related to the paranoid type of schizophrenia, namely, auditory hallucinations and persecutory delusions. Nonetheless, it may be of note that none of our toluene users showed avolition, alogia, inappropriate affect, stereotypy, mannerism, or social withdrawal, which are frequently seen in patients with schizophrenia. In general, these findings suggest that the toluene-related psychiatric condition may differ from that of schizophrenia.

In summary, we demonstrated an elevation of the Cho/Cr + PCr ratio in the basal ganglia of recreational toluene users, and this finding provides evidence of an abnormality in membrane metabolism. In addition, this membrane disturbance was associated with the emergence and persistence of psychiatric symptoms in toluene users. However, our results should be considered as preliminary due to the small sample size of the present study.

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REFERENCES

- American Psychiatric Association (1997). Practice guideline for the treatment of patients with schizophrenia. *Am J Psychiatry* **154**: 1–63.
- Ando K, Takei N, Matsumoto H, Iyo M, Isoda H, Mori N (2002). Neural damage in the lenticular nucleus linked with tardive dyskinesia in schizophrenia: a preliminary study using proton magnetic resonance spectroscopy. *Schizophr Res* **57**: 273–279.
- Arnold DL, Matthews PM, Francis GS, O'Connor J, Antel JP (1992). Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. *Ann Neurol* **31**: 235–241.
- Aydin K, Sencer S, Demir T, Ogel K, Tunaci A, Minareci O (2002). Cranial MR findings in chronic toluene abuse by inhalation. *AJNR Am J Neuroradiol* **23**: 1173–1179.
- Brand A, Richter-Landsberg C, Leibfritz D (1993). Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci* **15**: 289–298.
- Bromet EJ, Dew MA, Eaton W (1995). Epidemiology of psychosis with special reference to schizophrenia. In: Tsuang MT, Tohen M, Zahner GEP (eds). *Textbook in Psychiatric Epidemiology*. John Wiley & Sons: New York. pp 283–300.
- Bustillo JR, Lauriello J, Rowland LM, Jung RE, Petropoulos H, Hart BL et al (2001). Effects of chronic haloperidol and clozapine treatments on frontal and caudate neurochemistry in schizophrenia. *Psychiatry Res* **107**: 135–149.
- Chang L, Ernst T, Leonido-Yee M, Walot I, Singer E (1999). Cerebral metabolite abnormalities correlate with clinical severity of HIV-1 cognitive motor complex. *Neurology* **52**: 100–108.
- Cintra A, Aguirre JA, Andbjør B, Finnman UB, Hagman M, Agnati LF et al (1999). Subchronic toluene exposure in low concentrations produces signs of reduced dysfunction in the 6-hydroxydopamine lesioned nigrostriatal dopaminergic system of the rat. *Neurosci Lett* **274**: 5–8.
- Daniels DL, Houghton VM, Naidich TP (1987). *Cranial and Spinal MRI: An Atlas and Guide*. Raven Press: New York.
- Edelfors S, Ravn-Jonsen A (1989). The effect of toluene exposure for up to 18 months (78 weeks) on the (Ca²⁺/Mg²⁺)ATPase and fluidity of synaptosomal membranes isolated from rat brain. *Pharmacol Toxicol* **65**: 140–142.
- Escobar A, Aruffo C (1980). Chronic thinner intoxication: clinicopathologic report of a human case. *J Neurol Neurosurg Psychiatry* **43**: 986–994.
- Fenton WS, Hibbeln J, Knable M (2000). Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. *Biol Psychiatry* **47**: 8–21.
- Filley CM, Heaton RK, Rosenberg NL (1990). White matter dementia in chronic toluene abuse. *Neurology* **40**: 532–534.
- First MB, Spitzer RL, Gibbon M, Williams JB (1996). *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), Non Patient Version*. Biometrics Research. New York State Psychiatric Institute: New York.
- Flanagan RJ, Ives RJ (1994). Volatile substance abuse. *Bull Narcotics* **46**: 49–78.
- Fukuzako H, Fukuzako T, Takeuchi K, Ohbo Y, Ueyama K, Takigawa M et al (1996). Phosphorus magnetic resonance spectroscopy in schizophrenia: correlation between membrane phospholipid metabolism in the temporal lobe and positive symptoms. *Prog Neuropsychopharmacol Biol Psychiatry* **20**: 629–640.
- Gerasimov MR, Ferrieri RA, Schiffer WK, Logan J, Gatley SJ, Gifford AN et al (2002). Study of brain uptake and biodistribution of [¹¹C]toluene in non-human primates and mice. *Life Sci* **23**: 2811–2828.
- Hansson E, von Euler G, Fuxe K, Hansson T (1988). Toluene induces changes in the morphology of astroglia and neurons in striatal primary cell cultures. *Toxicology* **49**: 155–163.
- Heimberg C, Komoroski RA, Lawson WB, Cardwell D, Karson CN (1998). Regional proton magnetic resonance spectroscopy in schizophrenia and exploration of drug effect. *Psychiatry Res* **83**: 105–115.
- Hietala J, Syvalahti E, Vuorio K, Rakkolainen V, Bergman J, Haaparanta M et al (1995). Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. *Lancet* **346**: 1130–1131.
- Hillefors-Berglund M, Liu Y, von Euler G (1995). Persistent, specific and dose-dependent effects of toluene exposure on dopamine D₂ agonist binding in the rat caudate-putamen. *Toxicology* **100**: 185–194.
- Hormes JT, Filley CM, Rosenberg NL (1986). Neurologic sequelae of chronic solvent vapor abuse. *Neurology* **36**: 698–702.
- Huang J, Asaeda N, Takeuchi Y, Shibata E, Hisanaga N, Ono Y et al (1992). Dose[-]dependent effects of chronic exposure to toluene on neuronal and glial cell marker proteins in the central nervous system of rats. *Br J Ind Med* **49**: 282–286.
- Ives RJ (2000). Disorders relating to the use of volatile substances. In: Gelder MG, Lopez-Ibor JJ, Andreasen NC (eds). *New Oxford Textbook of Psychiatry*. Oxford University Press: New York. pp 546–550.
- Korbo L, Andersen BB, Ladefoged O, Møller A (1993). Total numbers of various cell types in rat cerebellar cortex estimated using an unbiased stereological method. *Brain Res* **609**: 262–268.
- Korbo L, Ladefoged O, Lam HR, Ostergaard G, West MJ, Arlien-Søborg P (1996). Neuronal loss in hippocampus in rats exposed to toluene. *Neurotoxicology* **17**: 359–366.
- Kozel N, Sloboda Z, Rosa M (1995). Epidemiology of inhalant abuse. An international perspective. *NIDA Res Monograph* **148**, NIH, Public #95–3831. Department of Health and Human Services: Rockville, MD (published online, at <http://www.drug-abuse.gov/pdf/monographs/148.pdf>).
- Kreis R, Farrow N, Ross BD (1991). Localized ¹H NMR spectroscopy in patients with chronic hepatic encephalopathy. Analysis of changes in cerebral glutamine, choline and inositols. *NMR Biomed* **4**: 109–116.
- Kyrklund T, Kjellstrand P, Haglid K (1987). Brain lipid changes in rats exposed to xylene and toluene. *Toxicology* **45**: 123–133.
- Ladefoged O, Strange P, Møller A, Lam HR, Ostergaard G, Larsen JJ et al (1991). Irreversible effects in rats of toluene (inhalation) exposure for six months. *Pharmacol Toxicol* **68**: 384–390.
- Lebel CP, Schatz RA (1990). Altered synaptosomal phospholipid metabolism after toluene: possible relationship with membrane fluidity, Na⁺,K⁺-adenosine triphosphatase and phospholipid methylation. *J Pharmacol Exp Ther* **253**: 1189–1197.
- Lundbom N, Gaily E, Vuori K, Paetau R, Liukkonen E, Rajapakse JC et al (2001). Proton spectroscopic imaging shows abnormalities in glial and neuronal cell pools in frontal lobe epilepsy. *Epilepsia* **42**: 1507–1514.
- Miller BL (1991). A review of chemical issues in ¹H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. *NMR Biomed* **4**: 47–52.
- Miller BL, Chung L, Booth R, Ernst J, Cornford M, Nikas D et al (1996). *In vivo* ¹H MRS choline correlation with *in vitro* chemistry/histology. *Life Sci* **58**: 1929–1935.
- Morton HG (1987). Occurrence and treatment of solvent abuse in children and adolescents. *Pharmacol Ther* **33**: 449–469.
- Neumark YD, Delva J, Anthony JC (1998). The epidemiology of adolescent inhalant drug involvement. *Arch Pediatr Adolesc Med* **152**: 781–786.

- Ohara K, Isoda H, Suzuki Y, Takehara Y, Ochiai M, Takeda H (2000). Proton magnetic resonance spectroscopy of lenticular nuclei in simple schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 24: 507–519.
- Overall JE, Gorham DR (1962). The Brief Psychiatric Rating Scale. *Psychol Rep* 10: 799–812.
- Petty RG (1999). Structural asymmetries of the human brain and their disturbance in schizophrenia. *Schizophr Bull* 25: 121–139.
- Pfefferbaum A, Adalsteinsson E, Spielman D, Sullivan EV, Lim KO (1999). *In vivo* brain concentrations of *N*-acetyl compounds, creatine, and choline in Alzheimer disease. *Arch Gen Psychiatry* 56: 185–192.
- Raikhlin-Eisenkraft B, Hoffer E, Baum Y, Bentur Y (2001). Determination of urinary hippuric acid in toluene abuse. *J Toxicol Clin Toxicol* 39: 73–76.
- Reynolds GP (1983). Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia. *Nature* 305: 527–529.
- Rosenberg NL, Grigsby J, Dreisbach J, Busenbark D, Grigsby P (2002). Neuropsychologic impairment and MRI abnormalities associated with chronic solvent abuse. *J Toxicol Clin Toxicol* 40: 21–34.
- Rosenberg NL, Kleinschmidt-DeMasters BK, Davis KA, Dreisbach JN, Hormes JT, Filley CM (1988). Toluene abuse causes diffuse central nervous system white matter changes. *Ann Neurol* 23: 611–614.
- Ross BM, Hudson C, Erlich J, Warsh JJ, Kish SJ (1997). Increased phospholipid breakdown in schizophrenia. Evidence for the involvement of a calcium-independent phospholipase A2. *Arch Gen Psychiatry* 54: 487–494.
- Sekine Y, Minabe Y, Kawai M, Suzuki K, Iyo M, Isoda H et al (2002). Metabolite alterations in basal ganglia associated with methamphetamine-related psychiatric symptoms: a proton MRS study. *Neuropsychopharmacology* 27: 453–461.
- Simmons ML, Frondoza CG, Coyle JT (1991). Immunocytochemical localization of *N*-acetyl-aspartate with monoclonal antibodies. *Neuroscience* 45: 37–45.
- Stanley JA, Pettegrew JW, Keshavan MS (2000). Magnetic resonance spectroscopy in schizophrenia: methodological issues and findings—Part I. *Biol Psychiatry* 48: 357–368.
- Tartaglia MC, Narayanan S, De Stefano N, Arnaoutelis R, Antel SB, Francis SJ et al (2002). Choline is increased in pre-lesional normal appearing white matter in multiple sclerosis. *J Neurol* 249: 1382–1390.
- Tsai G, Coyle JT (1995). *N*-acetyl-aspartate in neuropsychiatric disorders. *Prog Neurobiol* 46: 531–540.
- Unger E, Alexander A, Fritz T, Rosenberg N, Dreisbach J (1994). Toluene abuse: physical basis for hypointensity of the basal ganglia on T2-weighted MR images. *Radiology* 193: 473–476.
- Webb PG, Sailasuta N, Kohler SJ, Raidy T, Moats RA, Hurd RE (1994). Automated single-voxel proton MRS: technical development and multisite verification. *Magn Reson Med* 31: 365–373.
- von Euler G, Fuxe K, Bondy SC (1990). Ganglioside GM1 prevents and reverses toluene-induced increases in membrane fluidity and calcium levels in rat brain synaptosomes. *Brain Res* 508: 210–214.
- von Euler G, Ogren SO, Li XM, Fuxe K, Gustafsson JA (1993). Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D₂ agonist binding in the rat. *Toxicology* 77: 223–232.
- von Euler M, Pham TM, Hillefors M, Bjelke B, Henriksson B, von Euler G (2000). Inhalation of low concentrations of toluene induces persistent effects on a learning retention task, beam-walk performance, and cerebrocortical size in the rat. *Exp Neurol* 163: 1–8.