Prenatal Exposure to Influenza and Increased
Cerebrospinal Fluid Spaces in Schizophrenia

胎生期のインフルエンザ曝露と
分裂病者の脳脊髄液腔拡大との相関

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Noriyoshi Takei

**Prenatal Exposure to Influenza and Increased Cerebrospinal Fluid Spaces
in Schizophrenia**

Noriyoshi Takei, MD, MSc
Senior Lecturer

The Institute of Psychiatry
(University of London)
De Crespigny Park
Denmark Hill
London SE5 8AF
United Kingdom

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                       University of Tokyo

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Contents

during mid-gestation.

SUMMARY

Several epidemiological studies have suggested that maternal exposure to influenza during mid-gestation is a risk factor for schizophrenia. In exploring the possible pathogenic mechanism, we examined the relationship between CT structural brain measures in 83 schizophrenics and 113 controls, and their risk of maternal exposure to influenza. Four brain measures of the cerebrospinal fluid spaces (the lateral ventricle, maximum third ventricle, sulcal fluid, and sylvian fissure) were investigated in relation to the risk exposure level. In schizophrenics, sylvian fissures were found to increase with increasing level of risk exposure to influenza during the susceptible period, i.e., mid-gestation; no such effect was found in controls. These indicate that risk for mid-gestational influenza exposure is associated with an enlargement of the cerebro-spinal fluid spaces in the region of the temporal lobe. The findings suggest that the brain morphological abnormalities frequently reported in schizophrenics may in part be attributable to antenatal exposure to influenza.

I. BACKGROUND AND AIMS

Brain imaging studies, based on computed tomography (CT) and magnetic resonance imaging (MRI), have consistently reported that schizophrenics have enlarged ventricles, sulcal widening, and reduced volume of the temporal lobe (Shelton and Weinberger 1986; Lewis 1990; Andreasen et al. 1990a).

These abnormalities are present at the onset of illness (Weinberger et al. 1982; Schulz et al. 1983; Nasrallah et al. 1986; Illowsky et al. 1988; Bogerts et al. 1990), and many authorities believe that they are neurodevelopmental in origin, a view supported by neuropathological investigations (Roberts. 1991; Akbarian et al. 1993). Suddath et al. (1990), who examined 15 sets of monozygotic twins discordant for schizophrenia using MRI, found smaller volume of the temporal lobe gray matter, especially in the left temporal lobe, in 13 of the affected twins than in their unaffected cotwins, indicating that their cause is at least in part non-genetic.

Some recent epidemiological studies suggest that maternal exposure to influenza during mid-gestation may be a risk factor for later schizophrenia in the unborn child (Mednick et al. 1988; Barr et al. 1990; O'Callaghan et al. 1991; Sham et al. 1992; Adams et al. 1993; Takei et al. 1994; Mednick et al. 1994; Kunugi et al. 1995; Takei et al. 1995; Takei et al. 1996). However, the association between

prenatal exposure to influenza and schizophrenia remains at the epidemiological, indeed largely ecological level. The mechanism of the putative effect of maternal influenza remains obscure, and in the absence of such a plausible mechanism, scepticism about the hypothesis is natural (Crow and Done 1992).

As a first stage in exploring possible pathogenic mechanisms, this study attempted to investigate the relationship between structural neuroimaging findings in schizophrenic patients and their risk of prenatal exposure to influenza. It was hypothesised that schizophrenic patients whose mothers had been exposed to influenza epidemics during the susceptible period in mid-gestation would be especially likely to have abnormal brain morphology.

II. METHODS

Samples

Cases of functional psyhosis were drawn from two cross-sectional samples of consecutive hospital admissions, the Camberwell Collaborative Psychosis Study (Jones et al. 1993) and a sample drawn from an adjacent health district (Harvey et al. 1990). Sampling procedures were virtually identical for both studies. All hospital admissions aged 16 to 60 years were screened and recruited if delusions, hallucinations or formal thought disorder, as defined in the Research Diagnostic Criteria (RDC, Spitzer et al. 1978), were present without evidence of focal

neurological disease, or other organic cause such as epilepsy, drug abuse or alcoholism. Initial diagnostic assessment included the Present State Examination (Wing et al. 1974) and case note review. Subjects were included in the present study if they were born in the U.K., met the RDC criteria for schizophrenia, and, having given informed consent, underwent CT scanning (N=83).

Controls comprised unpaid volunteers actively recruited from three main sources: a Salvation Army training college in South London, a local Job Centre and the employees of the Institute of Psychiatry and associated hospitals. Inclusion criteria for volunteers were the same as for the patients, other than that controls were eliminated if there was evidence, from a semi-structured interview, of any psychiatric condition fulfilling the RDC definitions including alcoholism (N=67). Patients with an RDC diagnosis of schizo-affective disorder (N=24), mania or bipolar disorder (N=26), or major depression (N=12) were included as a further comparison group. The reasons for those patients with schizoaffective disorder to be included in the comparison group draw upon observations suggesting that from a familial perspective, this disorder is closely linked to affective illness rather than schizophrenia (Kendler et al. 1993a; 1993b) and that the potential risk effect of influenza may be associated with typical schizophrenia (Takei et al. 1995; 1996). Sampling procedures for this additional comparison group with psychiatric illness were identical to those described above for schizophrenic subjects.

CT Scan Measures

In this explanatory analysis, a historical data of CT scans was used since, at the time when the study was initiated, magnetic resonance imaging (MRI), which provides resolution superior to CT scanning, was not available to us. Axial CT scans were performed on a Siemens 9800 scanner between 1987 and 1992. Parallel to the floor of the anterior fossa and ascending to the vertex, 1 cm thick slices were examined at an independent viewing console (IVC). Raters (S.L., P.J., and I.H.) remained blind to the study hypothesis and the status of patient or volunteer; inter-rater reliability was high (intraclass correlation, r, >0.9 for each measure among raters). The volume measurements of intracranial region and lateral ventricle (LV), and area measurements of maximum third ventricle (3V), sulcal fluid (SF), and left and right sylvian fissures (SY) were used in the present study.

Intracranial area was measured on each slice by summing all pixels in the range 0 to 100 Hounsfield units (HU; Hounsfield. 1973) within the skull. The brain-cerebrospinal fluid (CSF) boundary of the sylvian fissures, third and lateral ventricles was traced manually, and the IVC required to sum pixels 0 to 25 HU within this area. An estimate of intracranial volume was made by adding together the intracranial areas of four slices; the most superior slice on which the anterior horns alone were visible, the slice below this and the two slices above. These slices yielding a truncated intracranial volume were hereafter referred to as simply

"intracranial volume". Areas of the sylvian fissures, and the lateral and third ventricles were measured in all slices where they appeared. We preferred the area measure for the third ventricle since the superior and inferior limits of the third ventricle can be difficult to define clearly in some subjects so that a single cross-sectional area taken from a middle slice can be regarded as the more valid measure (Jones et al. 1994). The measure of LV volume was constructed by adding together all the area measurements of its body, occipital horns, and frontal and anterior horns. Sulcal area was measured, using the most superior slice that still contained lateral ventricular fluid, by tracing manually within the skull and cerebrum, an annulus that contained the entire cortical outline and sulcal spaces. The IVC summed all pixels between 0 and 25 HU inside this trace to give an estimated area of sulcal fluid. Right and left sylvian fissures were defined manually, taking the most superior slice where the anterior horns were visible without the occipital horns or body of the LV coming into view.

A global visual rating on a 4-point scale (0-3) was also made, in an independent manner (the rater, P.J., remained blind to the identification of subjects and also to the quantitative assessments), of the SF and SY among the four brain regions (LV, 3V, SF, and SY) under investigation using reference photographs for each rating. These independent data of qualitative assessments were also used for analysis to see if the results obtained from the quantitative analyses remained consistent. Definitive or gross enlargement was given a score of 2 or above, but, in reality,

none of the schizophrenic subjects in the study were rated over 2 on the SY measures. For the purpose of this study, a rating of one (minimal enlargement) by the raters was taken as the presence of enlargement. However, for the SF measures, another cut-off point of 2 was also applied to the data so as to dichotomise the qualitative measurement as 11% of the sample had a score above this value.

Raters who made quantitative and qualitative assessments of brain morphology were also blind to any clinical characteristics of patients (e.g., diagnosis, date of birth).

Definition of Prenatal Exposure to Influenza

The crucial period of exposure to influenza was defined as 5 months before birth, corresponding to the 5th month of gestation. This definition relied upon previous studies reporting an increased risk of the disease when exposed to influenza during mid-gestation (Mednick et al. 1988; Barr et al. 1990). In particular, our own studies based on English samples like the subjects in this study indicate that those unborn babies exposed to influenza epidemics during the 5th month of gestation are at greater risk of schizophrenia (O'Callaghan et al. 1991; Sham et al. 1992; Takei et al. 1994). Any misclassification of exposure arising from the definition of vulnerable period in this study would bias the results towards the null hypothesis.

Next, the reported monthly number of deaths from influenza in England and Wales was used as a measure of the magnitude of risk exposure to influenza. For each subject, the number of deaths attributable to influenza during the 5th month of gestation was identified referring to the data on the reported deaths in the relevant month from the Registrar General's *Annual Reviews of Statistics for England and Wales*. The measures of risk exposure, i.e., number of deaths, were used for initial examination, but numbers of the influenza deaths identified for each subject were grouped into quartiles to facilitate subsequent analyses, yielding 4 "flu exposure" levels of very low, low, high, and very high. The 25%, 50%, and 75% cut-off number of deaths per month were 21, 64, and 206, respectively. For instance, if the number of deaths from influenza that occurred in the month 5 months before birth of a subject was less than 21, then he/she was classified as belonging to the lowest quartile of risk exposure to influenza.

Ideally, antibody titers of influenza in each patient's mother which are measured during the susceptible period of gestation would serve as the index of exposure level. Since it is impractical to obtain such information, we decided to use the above statistical data which were best available to us. It is, however, improbable that all patients included in the study as "very high" risk group had mothers who were actually infected with influenza. Rather, the proportion of those individuals included in the study whose mothers were infected when they were *in utero* would

increase according to the relative rate in the general population. The idea of this study was that if brain morphology in patients was characterised or distinguished by the current proxy measures of exposure to influenza, one could infer that brain morphological changes are related to prenatal exposure to influenza. Because of the non-normal distribution, all brain measures (LV, SF, and SY) except the 3rd ventricular area (3V) were processed in logarithmic (natural logarithm) transformation to allow the use of parametric statistical methods. Stem & leaf plots for the distribution of these four brain measures are shown in Fig 1.

III. STATISTICAL ANALYSIS

Initially, log-transformed brain measures in schizophrenics were regressed by the influenza deaths (in logarithmic scale) occurring 5 months before birth; the distribution of influenza deaths is shown in Fig 1. Subsequently, analysis of covariance (ANCOVA) was conducted, in which brain measures were taken as a variable of main interest and "flu exposure" level as a group variable. Intracranial volume (not log-transformed), age at CT (years), and duration of illness (years; age-of-onset minus age-at-CT scanning) were first included in the model as covariates. However, only intracranial volume among these three covariates was found to significantly correlate with the brain measures, and was therefore retained in the model throughout the analyses. In ANCOVA, several factors such as sex, ethnicity (white vs non-white), social class (class I-III vs class IV and V), season

of birth (January-April, May-August, and September-December) and birth period (birth before or after 1960) were allowed for as potential confounders when the factor of main interest, i.e., flu exposure level, was being examined. As our specific hypothesis was that there would be more cases of schizophrenia with brain abnormalities for high risk versus low risk exposure groups, we tested the polynomial contrasts of the brain measures according to the level of flu exposure. Prior to examining each brain region (i.e., LV, 3V, SF, and SY) separately by applying ANCOVA, a set of these four brain measures was taken as dependent variables, and any trend (i.e., enlargement in the CSF space according to the level of risk exposure) was explored using MANOVA. This was done to allow us to control for possible correlations between brain measures. Wilk's λ derived from MANOVA analysis was transformed to refer to the F-distribution with appropriate degrees of freedom.

Even if there were linearly increased brain measures with an increase in risk exposure level especially in the schizophrenic group, this could be due to a relative reduction in the measures in schizophrenics from low risk exposure categories (i.e., there would be no enlargement of CSF spaces among the higher risk exposure subgroups). Therefore, brain measures (SY) were compared between different groups using t tests; i.e., schizophrenia vs a comparison group, and additionally, schizophrenia vs normal volunteers alone.

When examining the dichotomous outcome of enlarged vs normal sulci, we used logistic regression to see whether enlarged SF and SY areas were related to the risk exposure to influenza. In this regression model, sex, ethnicity, paternal social class, season of birth, and birth period were allowed for as potential confounders except for the intracranial volume.

These procedures were implemented by statistical packages of Statistical Packages for Social Science (SPSS inc 1990) and EGRET for qualitative assessment (SERC 1991).

IV. RESULTS

The socio-demographic details of the cases and control groups are shown in Table 1. There was a higher proportion of males in the schizophrenic cases relative to the combined controls. The comparison groups contained more Caucasian subjects than the cases although, in this age group, the proportion of such subjects in the controls was similar to that found in the local district. The paternal socio-economic status in the normal controls was higher than that in the cases. The schizophrenic subjects were younger compared to the comparison groups, in particular to the combined controls.

Quantitative Analysis

Patients

83 U.K. born individuals met the RDC definition of schizophrenia and underwent CT scanning. Using a regression approach, initial examinations were made to see whether an increased level of risk exposure, the number of influenza deaths during the 5th month of gestation, would correspond to an enlargement in the CSF regions. Total sylvian fissure (SY) area measures in logarithmic scale were found to correlate significantly with an increase in the number of influenza deaths during the fifth month of gestation (log-transformed influenza deaths); scatter plot and fitted regression line are shown in Fig 2. None of the other regions under study (i.e., LV, 3V, and SF) approached statistical significance.

The four CT measures in relation to level of the exposure to influenza during mid-gestation are shown in Table 2. In these descriptive statistics, median values are presented for the LV, SF, and SY measures because these measures had a skewed distribution as mentioned earlier. It can be seen that the SY increases with the level of influenza exposure; the median area measure in the very high exposure level was 0.59 cm^2 (ranging from 0.00 to 4.02 cm^2), a more than two-fold increase in comparison with that for the very low exposure level (median 0.20 cm^2 ranging from 0.00 to 1.04 cm^2). A similar trend was also present, though to a lesser extent, in the SF area measures. Indeed, it is noticeable that the largest CSF area or volume measure was observed among the very high risk exposure group across all the four regions investigated.

However, we regarded these results as crude measures since no potential confounders were allowed for. Therefore, MANOVA & ANCOVA approaches were subsequently utilised to control for confounding factors. In these analyses, no significant effect of age at CT scanning or duration of illness (as covariates) was found on any of the four measurements examined. These two variables were not retained in the model since variation in these aspects of brain morphology in schizophrenics did not appear to correlate with age or length of illness. In the MANOVA model, the four measures were set as dependent variables and factors of sex, ethnicity, social class, season of birth, and birth period were taken into account along with intracranial volume as a covariate. The result for the trend for brain morphology with respect to the level of risk exposure to influenza revealed a significant linearity ($F_{1,25}$=3.66, p=.018) and some evidence of presence of additional quadratic association ($F_{1,25}$=2.62, p=.059).

Then, the four brain measures were analysed separately using ANCOVA. The results are given in Table 2. There was no significant linear trend in the region of lateral ventricle volume ($F_{1,39}$=0.05, p=.82) or maximum third ventricle area ($F_{1,39}$=1.98, p=.17), even after adjustment for potential confounders. However, a highly significant linear trend was present in the SY area measures ($F_{1,39}$=11.65, p=.002); that is, individuals with schizophrenia showed enlargement of SY area with an increased level of risk exposure to influenza. The parameter estimate and standard error of the risk exposure to influenza, allowing for intracranial volume

and confounders, indicate that there was a 0.29 cm^2 increase in SY area with one level increase in the risk exposure category (95% confidence interval, CI, 0.12 to 0.49 cm^2). The (geometric) means (95% CI) with adjustment for intracranial volume were 0.28 (0.12 to 0.46), 0.28 (0.13 to 0.45), 0.41 (0.23 to 0.61), and 0.62 cm^2 (0.42 to 0.86) for very low, low, high, and very high risk exposure level, respectively. For the SF area, there was also a significant trend ($F_{1,39}$=4.52, p=.042). No further polynomial terms, i.e., quadratic or cubic term, were significant for either SY or SF areas.

Because of these findings, the SY area measures were further examined by left and right side separately. Asymmetric measures in SY area (see the footnote in Table 3) were also investigated. There was a highly significant trend in both left ($F_{1,39}$=10.10, p=.004) and right ($F_{1,39}$=10.23, p=.003) SY according to the level of risk exposure to influenza when potential confounders were allowed for. However, there was no particular relationship between asymmetric indices of SY area measures and risk exposure to influenza ($F_{1,39}$=0.00, p=.98).

Comparison Groups

Initially, the normal volunteer and psychiatric (other than schizophrenic) patient groups were compared with respect to brain measures. No significant differences were found in any of four brain regions between these two. As the number of normal volunteers in our sample was limited, we therefore included patients with

a diagnosis of schizo-affective disorder, mania or bipolar disorder, or major depression, as well as normal volunteers, into a single group to compare against schizophrenics.

The same MANOVA analysis was then conducted for this large comparison group. 113 individuals, including 56 normal volunteers, had the complete information required for the analysis. The level of risk exposure to influenza used for schizophrenics was similarly applied to these subjects. The results obtained from the MANOVA revealed that there was no linear significant trend of brain measures over the level of risk exposure ($F_{4,99}$=0.27, p=.89). In further ANCOVA analyses, no significant trend was observed for any of the four measures; $F_{1,102}$=0.37 (p=.55) for LV volume, $F_{1,102}$ =0.00 (p=.95) for 3V area measures, $F_{1,102}$=0.42 (p=.52) for SF area measures, and $F_{1,102}$ =0.18 (p=.68) for SY area measures. When these procedures (MANOVA & ANCOVA) were further applied to the data on normal volunteers alone, no significant trend was detected for any of the measures including the SY area ($F_{1,45}$=0.50, p=.49).

Comparisons of Sylvian fissure Area Measures in the Risk Exposure Categories between Schizophrenics and Others

The results from t tests (Table 4) showed that the geometric mean (0.65 cm^2) of SY area measures for those schizophrenics who were categorised as the very high risk exposure subgroup, differed from that in the same risk exposure level for both

the combined comparison group (0.39 cm²; t=1.91, df=56, p=.062) and the normal volunteers alone (0.26 cm²; t=2.52, df=34, p=.017). In none of other exposure levels (very low, low, and high), did significant differences emerge; 95% CI of differences in SY area measures among different groups are shown in Table 4.

Qualitative Analysis

Then, the qualitative assessment of the abnormalities in the SF and SY area was examined. The proportion showing abnormalities in the SY region increased with the level of risk exposure to influenza (see Table 5); the (unadjusted) odds ratio (OR) of enlarged vs normal increased from 1 (reference), 1.19, 2.43, to 3.05. However, this failed to reach a conventional significance level (χ^2 trend=2.91, df=1, p=.088). After adjustment for confounders, no significant trend was again detected (log-likelihood ratio statistic, LRS, =2.03, df=1, p=.16), but the adjusted estimate of odds ratio further increased in the very high risk exposure category (adjusted OR=5.36, 95% CI 0.48 to 59.40); because of the small sample size involved, the confidence interval was, however, very wide. For the SF area, a slightly elevated OR of 1.39 (95% CI 0.17 to 11.25) was observed in the very high exposure level, but no significant trend was present; LRS=0.04, df=1, p=.84. In these analyses, a cut-off point of one (4 point scale, 0-3, on the qualitative assessment of the enlargement) was used for the reason mentioned earlier.

When another cut-off point of 2 was used for dichotomising the qualitative

assessment of the SF region (not applicable to the data on SY, see **Methods**), and the frequencies of cases with enlargement were examined in relation to the level of influenza exposure, a χ^2 test for trend fell short of statistical significance (χ^2=3.25, df=1, p=.071); unadjusted ORs increased from 1 (very low; as reference) through 0.86 (low), 3.35 (high), to 4.75 (very high exposure level). Logistic regression analysis was not conducted further because of the small number of patients designated as having enlarged SF with use of this cut-off point.

V. DISCUSSION

Our study suggests that presumed risk of maternal exposure to influenza epidemics during mid-pregnancy is associated with enlargement of the sylvian fissure in schizophrenia. This implies that schizophrenics exposed to a high prevalence of influenza during the critical period of fetal development have a higher likelihood of having abnormalities in the region of the temporal lobe than normal.

Methodological issues

Before accepting these findings we need to establish whether there are other interpretations. Firstly, when many brain areas are investigated, this increases the probability of finding an effect by chance in some area measures. However, even

when the fact that we examined four brain areas (i.e., lateral ventricle, third ventricle, sulcal fluid, and sylvian fissure) is taken into account, the p value of 0.002 for the sylvian fissure area measures associated with the risk exposure to influenza is much lower than that (0.05/4=0.0125) required after Bonferroni adjustment. If the brain measures correlate each other, each test (ANCOVA) is no longer regarded as independent. To guard against this problem, we initially conducted MANOVA, in which the possible correlations among the brain measures were allowed for, revealing a significant trend (p=.018) of CSF space enlargement in relation to an increase in the influenza exposure level.

Has any systematic bias in this study contributed to the results? If the raters, who assessed the brain structure, had known the hypothesis and the level of exposure status in each individual with schizophrenia, this could have biased the results in favour of the study hypothesis. However, the raters involved in the assessment of brain morphology completed the measurements prior to the hypothesis being put forward and, moreover, were kept completely blind to case-control status and any clinical characteristics of patients. It is extremely difficult to imagine that patients were selected in any biased manner so as to yield the current results. Brain morphological changes were compared within patients with the disease in relation to the risk exposure level. Had the subjects with schizophrenia been compared with normal volunteers or those with other psychiatric disorders, bias in selecting procedures, if any, might have played a role in the findings. On the

contrary, random misclassification of exposure status and measurement error of brain structure in both qualitative and quantitative assessments may have taken place, serving to obscure rather than to exaggerate the effect.

However, questions about measurements in the CSF spaces, in particular the intracranial volume, may be raised. We did not use the Cavalieri principle to estimate intracranial volume, simply because we used an archived data set for our exploratory analysis of our hypothesis and the relevant software was, unfortunately, unavailable locally. It is, therefore, possible that our estimates of intracranial volume are biased. In the present study (quantitative analysis), these measures were allowed for as a covariate on the grounds that one with a large head has large CSF spaces, notably the ventricles. If the intracranial volume was coincidentally underestimated in schizophrenics who were classified into the most vulnerable group (very high influenza exposure group), then the results would have been biased in favor of hypothesis. However, the mean of the estimated intracranial volume in this group (652.08 cm^3) was relatively larger than the average in the whole sample (641.92 cm^3, see Table 1). Moreover, the measurements of intracranial volume were, in fact, highly correlated with the ventricle measurements ($r=0.319$, $p<.01$, for the lateral ventricle and $r=0.412$, $p<.002$, for the maximum third ventricle), but least so for the measures of the sylvian fissures ($r=0.203$, $p>.10$).

If potential confounding factors were not sufficiently taken into account, erroneous conclusions might have been reached. As sex, ethnicity, and socio-economic status are related to the size of extra- and intracranial CSF spaces (Williams et al. 1985; Owens et al. 1985; Jones et al. 1994; van Os et al. 1995), failure to control for these potential confounding effects could produce spurious results. In reality, these factors cannot however be regarded as confounders, unless the risk exposure level, i.e., prenatal exposure to influenza, is independently linked with these factors. Under the circumstances where it is difficult to judge whether some factors really confound the effect, it is much safer to take them into account. In addition to these potential confounders, we further allowed for the factors of season of birth and birth period in MANOVA & ANCOVA analyses. Even after adjustment for all of these, the area measures of sylvian fissures were found to significantly correlate with the risk exposure level of influenza during mid-gestation. Furthermore, in the assessment of qualitative measurements, the adjusted estimate of the odds ratio of having an enlarged vs normal sylvian fissure in the very high risk exposure level against the very low risk level, was more marked after these confounders were controlled for. Thus, our findings cannot be attributed to confounders. Rather, any effect arising from confounders minimised rather than accentuated the relationship between the brain area measures and the risk exposure to influenza. Could the present findings be accounted for by treatment effects such as ECT (electrroconculsive therapy) and dosage of neuroleptics? Many studies have shown that brain morphological changes are

present in first-episode schizophrenic patients (Weinberger et al. 1982; Turner et al. 1986; Illowsky et al. 1988) and that these abnormalities are non-progressive (Vita et al. 1988; DeLisi et al. 1992). In effect, no significant relationship was, in this study, evident between brain measures, in particular sylvian fissure, and duration of illness. Furthermore, it is difficult to imagine that the choice of treatment is influenced by the the level of exposure, i.e., number of deaths from influenza during midgestation. However, there may still be an unknown confounding factor that can instead explain the present findings, but it should be correlated with both the risk of maternal influenza infection and brain morphological changes in their offspring.

It is certainly true that the results from the qualitative assessment of sylvian fissures did not reach statistical significance. Since the small sample size leads to lack of power when several variables are included in the logistic regression analysis, feasibility of this attempt is naturally put in doubt. Nevertheless, the results showing that the relative risk of enlargement in the sylvian fissure area among the "very highly" exposed group is 5.4 (95% CI 0.5 to 59.4) in comparison with the reference group, are at least consistent with the findings obtained in the quantitative examinations.

To define the timing of risk exposure to influenza during gestation, we relied upon dates of birth and assumed full term delivery in each individual under study. This

may have caused misclassification of risk exposure level since an unknown proportion of subjects may have been born prematurely or postmaturely. This problem, however, would underestimate rather than exaggerate the relationship of interest.

The study is, however, not totally immune from criticisms; one may argue that the definition of risk exposure levels is not based on a direct measurement of antibody titers in mothers whose fetus later developed schizophrenia. It is impractical for us to obtain such data and thus we chose a correlational approach in this study. Nevertheless, as discussed earlier, such misclassification of exposure status would bias results towards the null hypothesis. However, some cases among the total 83 schizophrenics of our sample may be related etiologically to prenatal exposure to influenza (Sham et al. 1992; Takei et al. 1995), and these presumably come from the higher risk exposure groups.

We have considered possible alternative explanations for the present findings, arising from chance, bias, and confounding factors, and find it difficult to conclude that these contributed to the findings. Moreover, no similar correlations were observed when the risk of maternal exposure to influenza 2 months before birth (as an example of the third trimester exposure) and 8 months before birth (as an example of the first trimester exposure) was examined in relation to our four brain morphological measures in the same sample of schizophrenia; there was no

significant increase in the CSF spaces with an increase in the risk of maternal exposure to influenza (i.e., number of deaths from influenza) during either of first trimester exposure (MANOVA, $F_{4,25}$=2.04, p=.12) or third trimester (MANOVA, $F_{4,30}$=0.28, p=.89), thereby further strengthening our specific hypothesis of the risk effect of the *second* trimester exposure to influenza on the risk of schizophrenia.

It should also be noted here that no such association was present in the normal controls. To be sure that the results of non-significance for the normals were not due to lack of statistical power, we further examined the relationship using the comparison group consisting of normals and patients with psychosis other than schizophrenia combined, and again there was no evidence suggesting that CSF spaces increase with an increase in the level of prenatal influenza exposure in this large comparison group.

Implications

First episode CT studies (Turner et al. 1986) and follow-up studies (Nasrallah et al. 1986; Illowsky et al. 1988; Vita et al. 1988) have shown that ventricle size is not related to duration of illness. In this study, as in our previous study of the larger sample from which the present subjects were drawn (Jones et al. 1994), we found no association between the length of the disease process and brain structural changes. Recent magnetic resonance imaging (MRI) studies also found no

progressive changes in the brains of schizophrenic patients (Degreef et al. 1991; DeLisi et al. 1992). The lack of progression and presence of the brain abnormalities at first episode, raises the possibility that in schizophrenia ventricle size is determined early in life before manifestation of illness. Several investigators have examined the well replicated findings of a winter/spring birth excess in schizophrenia in relation to structural brain abnormalities. Two studies showed no association between season-of-birth effect and ventricle size in schizophrenia (Degreef et al. 1988; Pearlson et al. 1989), whilst two others did (Zipursky and Schultz 1987; Sacchetti et al. 1992).

Although no consensus has so far emerged, it is likely that early environmental hazards during neurodevelopment contribute to the brain morphological changes found in schizophrenia. However, why should prenatal exposure to influenza be associated with enlargement of the sylvian fissure but not the other brain regions investigated in this study? Lateral ventricular enlargement has been the most consistently demonstrated anomaly in schizophrenia (Shelton and Weinberger 1986; Andreasen et al. 1990b; Suddath et al. 1989; Lewis 1990), and there have been some suggestions that it is associated with perinatal complications (Murray et al 1985; Cannon et al 1993). Therefore, our findings of enlarged sylvian fissures were not surprising, and are compatible with the decreased volume of the temporal lobes frequently reported in schizophrenics.

It could be, however, argued that the demonstrated association between *in utero* exposure to influenza and enlarged CSF spaces in schizophrenia in this study could be accounted for by other variables which are related to the prevalence of influenza such as concurrent viruses or bacteria, or remedies against infection. We previously examined any relationship between 16 infectious diseases other than influenza and risk of developing schizophrenia, and found that only increased national deaths from bronchopneumonia, which are known to accompany influenza epidemics (Curwen et al. 1990), preceded, by 3 to 5 months (equivalent to the influenza vulnerable period), an increased number of preschizophrenic births (O'Callaghan et al. 1994).

A recent prospective study that examined the relationship between brain morphology and outcome in functional psychoses, found that larger sylvian fissures are associated with poor outcome (van Os et al, 1995), indicating that enlargement of the sylvian fissure in schizophrenia is an important clinicopathological index. The formation of the sylvian fissure is anatomically linked with the superior temporal gyrus. Recent MRI studies have indicated that the reduction in volume of the superior temporal gyrus is correlated with severity of auditory hallucination (Barta et al. 1990) and degree of thought disorder (Shenton et al. 1992). Intriguingly, this region is known to be formed during mid-gestation, about 21-23 weeks (Chi et al. 1977).

Our study needs to be interpreted with caution before the findings are replicated by other larger studies, preferably using magnetic resonance imaging. In view of unknown cause and mechanism of a morphological alteration in schizophrenic brains, such studies are warranted.

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Table 2. Area or volume measures of brain regions in schizophrenia in relation to exposure to influenza during the mid-gestation.

Influenza exposure level (Sample size)	Very Low (20)	Low (23)	High (20)	Very High (20)	Linear trend[†] (p value)
Median LV volume (cm^3) minimum, maximum	16.09 7.82, 39.69	18.39 5.27, 59.45	14.50 2.81, 62.35	18.84 6.72, 38.69	F=0.05 (.822)
Mean 3V area±SD (cm^2)	0.58±0.29	0.64±0.31	0.59±0.28	0.69±0.33	F=1.98 (.170)
Median SF area (cm^2) minimum, maximum	0.02 0.00, 0.98	0.10 0.00, 0.62	0.08 0.00, 1.31	0.14 0.00, 0.88	F=4.52 (.042)
Median SY area (cm^2) minimum, maximum	0.20 0.00, 1.04	0.22 0.00, 1.78	0.36 0.00, 1.51	0.59 0.00, 4.02	F=11.65 (.002)

[†]: Polynomial contrast (linear trend) while factors of sex, ethnicity, social class, season of birth, and birth period were adjusted for.

LV: lateral ventricle; 3V: maximum third ventricle; SF: sulcal fluid; SY: sylvian fissure.

Table 1. Sociodemographic characteristics and mean intracranial volume measures for the sample.

		Schizophrenia	Combined controls[1]	Normal controls
Size		83	113	56
Male/Female (% male)		60/23 (72%)	55/58 (49%)[*]	33/23 (59%)
Ethnicity	White	55	96[**]	51[***]
	Other	28	17	5
Parental social class[2]	I-III	55	87	50[†]
	IV-V	28	26	6
Season of birth (month)	1-4	33	39	18
	5-8	26	39	23
	9-12	24	35	15
Year of birth	<1960	26	65[‡]	28[§]
	1960 or later	57	48	28
Age at CT scanning (SD), yrs		27.53 (7.82)	32.74 (8.15)[∥]	31.77 (7.28)[¶]
Intracranial volume (SD), cm³		641.92 (45.88)	641.41 (51.39)	650.37 (50.45)

[1] Patients with a RDC diagnosis of schizo-affective disorder, mania or bipolar disorder, or major depression and normal controls.
[2] Social class according to parental occupation at birth or early childhood (Registrar General's classification).
[*] p=<.001 (χ^2=11.01, df=1; v. schizophrenia).
[**] p=.002 (χ^2=9.45, df=1; v. schizophrenia). [***] p<.001 (χ^2=11.37, df=1; v. schizophrenia).
[†] p<.002 (χ^2=9.59, df=1; v. schizophrenia).
[‡] p<.001 (χ^2=13.20, df=1; v. schizophrenia). [§] p=.027 (χ^2=4.91, df=1; v. schizophrenia).
[∥] p<.001 (t=4.50, df=194; v. schizophrenia). [¶] p=.002 (t=3.22, df=137; v. schizophrenia).

Table 3. Area measures of sylvian fissures in schizophrenia in relation to exposure to influenza during the mid-gestation.

Influenza exposure level (Sample size)	Very Low (20)	Low (23)	High (20)	Very High (20)	Linear trend[†] (p value)
Median left SY area (cm^2) minimum, maximum	0.14 0.00, 0.63	0.10 0.00, 1.17	0.18 0.00, 1.07	0.30 0.00, 2.63	F=10.10 (.004)
Median right SY area (cm^2) minimum, maximum	0.09 0.00, 0.53	0.06 0.00, 0.61	0.13 0.00, 0.65	0.19 0.00, 1.39	F=10.23 (.003)
Median asymmetric measure[‡] in SY area (cm^2) minimum, maximum	0.07 -0.33, 0.29	0.00 -0.23, 0.42	0.06 -0.31, 0.87	0.07 -0.51, 0.79	F=0.00 (.977)

[†]: Polynomial contrast (linear trend) while factors of sex, ethnicity, social class, season of birth, and birth period were adjusted for.
[‡]: This was calculated as follows: (left minus right sylvian fissure area measure / (total area measure of sylvian fissure x 1/2). Positive value implies that the area measure of left sylvian fissure is larger than that of right sylvian fissure.

SY: sylvian fissure.

Table 4. Comparisons of geometric mean for total sylvian fissure area measures (cm^2) in relation to exposure to influenza during the mid-gestation between diagnostic groups.

Influenza exposure level	Very Low	Low	High	Very High
Schizophrenia	0.27 (20)	0.28 (23)	0.39 (20)	0.65 (20)
Normals plus psychotics other than schizophrenics[†]	0.30 [-0.11 to 0.14] (19)	0.29 [-0.15 to 0.16] (33)	0.52 [-0.11 to 0.24] (26)	0.39 [-0.42 to 0.01]* (38)
Normals	0.28 [-0.15 to 0.16] (8)	0.28 [-0.18 to 0.21] (15)	0.62[‡] [-0.05 to 0.30] (17)	0.26 [-0.63 to -0.05]** (16)

[]: 95% confidence interval of difference in area measures compared with schizophrenic subjects.
(): Sample size.

*: p=.062 (2-tailed *t* test), **: p=.017 (2-tailed *t* test). In this analysis, log-transformed values were used.

[†]: Three subjects with missing values on some confounders were added for this analysis (a total of 116 subjects were used).
[‡]: This value is relatively high, although non-significant when compared with schizophrenic patients. This was due to the fact that 4 of 5 outliers (i.e., above geometric mean value plus 2 SD's) were incidentally clustered in this exposure group. Since an increase in sylvian fissure area measure was not hypothesised in this exposure level in control group, this relatively high value can be regarded as a chance phenomenon.

Table 5. Qualitative assessment of brain abnormalities in the sylvian fissure area in relation to risk exposure to influenza during mid-pregnancy.

Influenza exposure level	Very low	Low	High	Very high	Trend
Presence of enlargement	3	4	6	7	
Normal	17	19	14	13	
Unadjusted odd ratio	1*	1.19	2.43	3.05	
Adjusted odd ratio[2] (95% confidence interval)	1*	1.33 0.17, 10.72	2.28 0.27, 18.94	5.36 0.48, 59.40	LRS[1]=2.03, df=1 p=.155

*: reference category.
[1]: log-likelihood ratio statistic.
[2]: sex, ethnicity, social class, and season of birth, and birth period were controlled for.

Fig 1. Stem & leaf plots of four brain measures and influenza deaths as risk exposure for schizophrenics.

A. Brain measures

I) lateral ventricle volume (cm^3)

```
Cases (N)   Stem &  Leaf

   2         0  *   24
  11         0  *   56777888899
  18         1  *   011122222333344444
  20         1  *   55556666778888889999
  10         2  *   1222333444
  11         2  *   55567778889
   3         3  *   013
   5         3  *   57889
   1         4  *   2
   1         5  *   9
   1         6  *   2
```

Stem width: 10.0

II) maximum third ventricle area (cm^2)

```
Cases (N)   Stem &  Leaf

   2        0. *    11
  15        0. *    222222223333333
  27        0. *    444444444444555555555555555
  20        0. *    66666666667777777777
   7        0. *    8889999
   7        1. *    0000011
   3        1. *    222
   2        1. *    44
```


III) sulcal fluid area (cm^2)

```
Cases (N)   Stem &  Leaf

  42        0.0  *   000000000000000000000001112222334666677899
  15        0.1  *   000000114466779
   6        0.2  *   013458
   1        0.3  *   0
   5        0.4  *   00168
   2        0.5  *   04
   4        0.6  *   1235
   2        0.7  *   38
   2        0.8  *   28
   1        0.9  *   8
   1        1.0  *   9
   1        1.2  *   3
   1        1.3  *   1
```

IV) sylvian fissure area (cm^2)

Cases (N)	Stem & Leaf
31	0 . * 00000000000000000000000111111111
21	0 . * 222222222333333333333
11	0 . * 44444445555
4	0 . * 6677
6	0 . * 889999
5	1 . * 00111
4	1 . * 5889
1	4 . * 0

B. Influenza deaths

| Cases (N)	Stem & Leaf
32	0 * 00001111111111111111112222223333444
22	0 * 5555555566677778888899
4	1 * 3334
3	1 * 556
6	2 * 001444
3	2 * 578
1	3 * 4
1	4 * 4
2	5 * 01
2	6 * 45
1	7 * 5
1	8 * 8
1	15 * 7
1	25 * 7
1	31 * 5
1	32 * 4
1	35 * 2

Stem width: 100.0

Fig 2. Scatter plot of sylvian fissure area measures against the number of deaths from influenza during the fifth month of gestation.
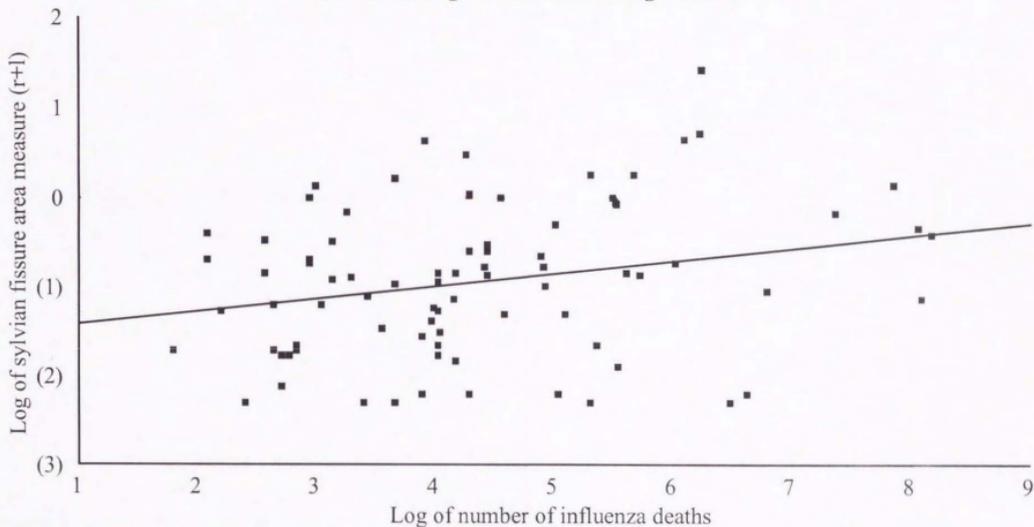
Sylvian fissure area measures and influenza
deaths were log-transformed.
Slope=0.141 (SE=0.061), p=.0239.

Kodak Gray Scale

A 1 2 3 4 5 6 **M** 8 9 10 11 12 13 14 15 **B** 17 18 19
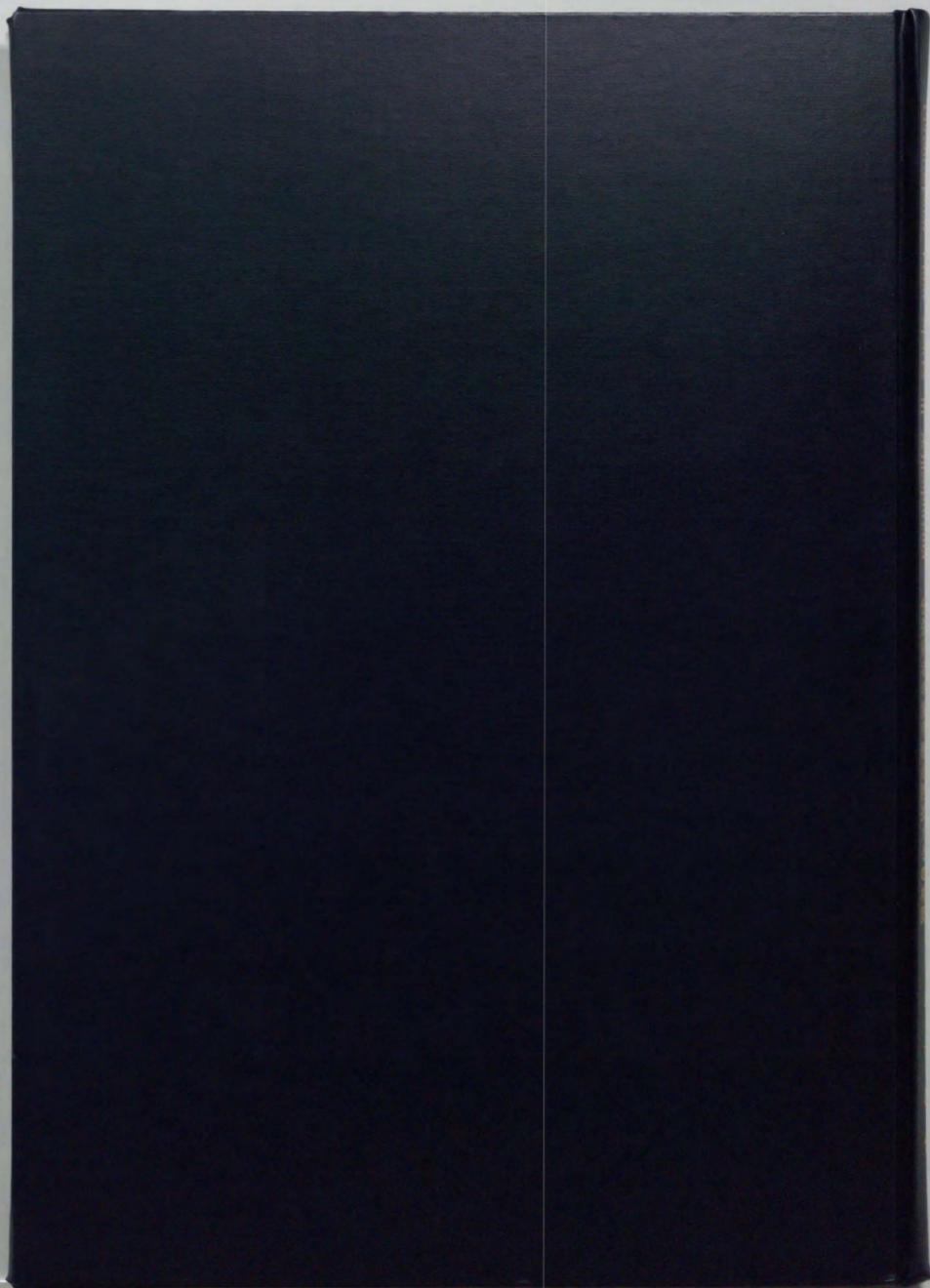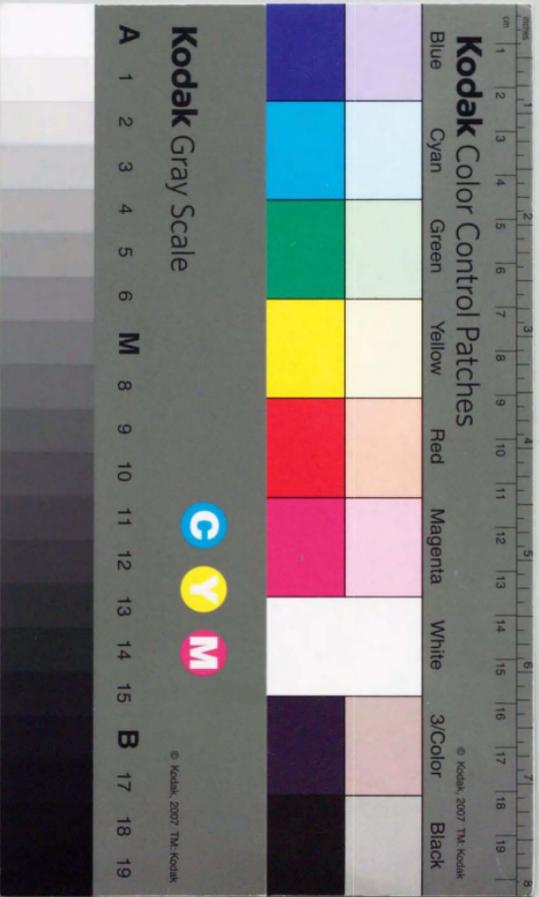
Kodak Color Control Patches

Blue Cyan Green Yellow Red Magenta White 3/Color Black

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C Y M