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17. A highly reproducible specific binding of 0.15 n*M* [^{125}I]iodosulpride representing 1.1 ± 0.1 and 0.6 ± 0.1 fmol per milligram of protein was detected in parietal cortex and cerebellum, respectively (corresponding values being 54.0 ± 2.5 in striatum and 2.0 ± 0.3 in frontal cortex) over a nonspecific binding defined with 25 μ*M* apomorphine corresponding to less than 20 percent of the total (filter blank deduced). In both regions, the pharmacology of these sites was studied with the dopaminergic and nondopaminergic compounds depicted in Fig. 1C for striatal sites. No significant difference in the three regions was found for the values of the median inhibition constant (IC_{50}) of the various agents (for example, the IC_{50} value of (−)-sulpiride was 11.8 ± 2.0, 12.0 ± 1.8, and 12.3 ± 2.3 n*M* and of (+)-sulpiride 495 ± 123, 423 ± 56, and 553 ± 188 n*M*, in the striatum, parietal cortex, and cerebellum, respectively). Moreover, the extremely high correlation between the three sets of IC_{50} values indicates that the sites labeled by [^{125}I]iodosulpride in the parietal cortex and cerebellum have a dopaminergic nature (slope, 0.998; $r = 0.989$, $P < 0.001$; slope, 1.036, $r = 0.996$, $P < 0.001$ between striatum and parietal cortex and between striatum and cerebellum, respectively) (M.-P. Martres, M.-L. Bouthenet, N. Salés, P. Sokoloff, J.-C. Schwartz, in preparation).
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Detection of Serum Antibodies to Borna Disease Virus in Patients with Psychiatric Disorders

Abstract. *Borna disease virus causes a rare meningoencephalitis in horses and sheep and has been shown to produce behavioral effects in some species. The possibility that the Borna virus is associated with mental disorders in humans was evaluated by examining serum samples from 979 psychiatric patients and 200 normal volunteers for the presence of Borna virus-specific antibodies. Antibodies were detected by the indirect immunofluorescence focus assay. Antibodies to the virus were demonstrated in 16 of the patients but none of the normal volunteers. The patients with the positive serum samples were characterized by having histories of affective disorders, particularly of a cyclic nature. Further studies are needed to define the possible involvement of Borna virus in human psychiatric disturbances.*

Borna disease virus causes a rare meningoencephalitis in horses and sheep in certain areas of Germany and Switzerland, where it has been endemic for over 150 years. The virus has not been classified, but because it may lead to persistent infections it is often considered to be a member of the slow virus group. The incubation period varies between a few weeks and several months. Characteristic symptoms of the disease are excitability or apathy, spasms, and partial paralysis. The disease is usually fatal (*1*).

The Borna virus has not been characterized biochemically. It replicates in a variety of cell lines after cocultivation with brain cells from infected animals. The virus persists in these cell lines and is noncytopathic. Intranuclear viral antigen can be demonstrated by immunohistology (*2*). Filtrates of brain homogenates from infected animals can be used to transmit the virus to a broad spectrum of animals ranging from chicken to chimpanzee, but the incubation periods and the clinical manifestations vary considerably. Whereas the course of the disease in some experimentally infected animals is similar to that observed in the natural disease of horses and sheep, in other species the disease remains subclinical or is evidenced only by behavior abnormalities (*1*, *3*, *4*). Behavioral changes resulting from Borna virus infection have been described in detail in the tree shrew *Tupaia glis* (*5*). The changes are manifested as a disinhibition toward the environment or, more specifically, as a reduction in cognitive ability. Infected tree shrews show a slight drowsiness and a disturbance in sexual behavior. Morphological studies implicate the limbic system in these alterations (*5*). Similar behavioral disorders occur in Borna virus–infected rats (*6*, *7*), in which a virus-specific cellular immune response can be demonstrated (*7*, *8*). Virus-specific antibodies can be demonstrated in the serum of infected animals by an immunofluorescence binding assay (*4*).

In view of the prominent central nervous system and behavioral effects produced by the Borna virus in experimentally infected animals, we wondered whether mental disorders in humans might, in some cases, be accompanied by the appearance of Borna virus–specific antibodies. To explore this possibility we obtained serum samples from 979 patients with emotional and depressive disorders from psychiatric clinics in the United States (Philadelphia) and in different areas of Germany (Giessen and Würzburg), and screened them for the presence of Borna virus–specific antibodies.

The patients in Philadelphia were attending the Depression Research Unit or the Lithium Clinic of the Hospital of the University of Pennsylvania. All of them were evaluated in a semistructured interview format, and diagnoses were assigned according to Research Diagnostic Criteria (*9*). Normal control subjects were obtained primarily from the hospital and university communities. They were evaluated in a similar semistructured interview format, and only those who were found to be free of significant medical illnesses, psychiatric disorders, or family histories of psychiatric illnesses were included in the study. Blood samples were obtained from a total of 285 patients with unipolar and bipolar depression and 105 normal healthy volunteers. The samples were centrifuged at 2500 rev/min for 15 minutes. The sera were then immediately frozen in coded tubes, in randomized order with respect to patients and healthy controls, and were shipped on dry ice to Giessen for analysis.

In addition, 686 psychiatric patients from Würzburg and eight patients from Giessen were evaluated, along with 95 control subjects. The patients were randomly selected from a heterogeneous population of hospitalized patients and represented a variety of psychiatric disturbances.

Antibodies were detected by the indirect immunofluorescence focus assay (*2*, *4*). Sera were diluted 1:10 in swine serum, absorbed with swine liver powder (100 mg/ml) to eliminate nonspecific background staining, and added in twofold dilutions to acetone-fixed Madin Darby canine kidney (MDCK) cells persistently infected with Borna virus strain He/80, originally isolated from a horse (*4*). Cells were incubated for 30 minutes at 37°C, washed in phosphate-buffered saline, and reacted with fluorescein isothiocyanate (FITC)-conjugated goat antiserum to human immunoglobulin G

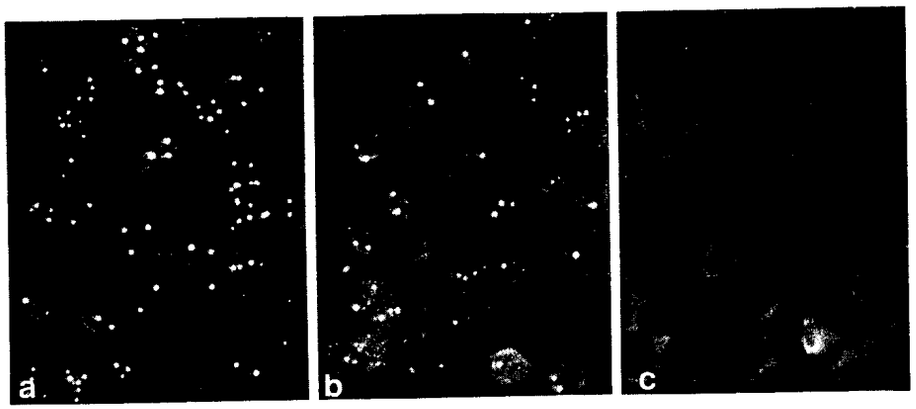

Fig. 1. Indirect immunofluorescence assay of MDCK cells persistently infected with Borna virus and exposed to (a) a human serum positive for Borna virus antibodies and (b) serum from a rat infected intracerebrally with Borna virus. (c) Uninfected MDCK cells treated with a positive human serum.

Table 1. Detection of Borna virus–specific antibodies in human sera by indirect immunofluorescence.

Serum samples from	Number of serum samples		Antibody titers of positive sera
	Total	Positive	
Philadelphia patients	285*	12	1:10 (3), 1:20 (4)† 1:40 (3), 1:80, 1:320
Giessen patients	8	1	1:40
Würzburg patients	686	3	1:20
			1:20
			1:40
Total	979	16	1:20
Control subjects in United States and Germany	200	0	

*Serum samples from each of four patients were checked twice with the same results. †Numbers in parentheses refer to number of samples showing a given titer.

(Miles-Yeda, Jerusalem, Israel) for 30 minutes. Uninfected MDCK cells and sera from Borna virus–infected rabbits or rats reacting with the appropriate species-specific IgG systems were used as positive controls.

Antibodies to Borna virus were present in serum samples from 12 patients from Philadelphia but none of the control subjects (Table 1). The samples were positive at antibody titers ranging from 1:10 to 1:320. Additional serum samples were obtained from four of the patients with positive antibody titers. These repeat samples, assayed under blind conditions, were all positive for Borna virus antibody.

The 12 patients with positive antibody titers included six males and six females. Their ages ranged from 21 to 63 years, with a mean age of 42.3 ± 3.8 (standard error of the mean) years. Seven patients had a diagnosis of primary unipolar depression and five were diagnosed as having bipolar depression.

Three of the patients from Würzburg and one from Giessen were positive for Borna virus antibodies, with titers ranging from 1:20 to 1:40. These four patients showed emotional disorders characterized by depression, apathy, and disorganization. The 95 control subjects from Würzburg and Giessen showed no antibody to Borna virus.

When exposed to human serum containing antibodies to Borna virus, the Borna virus-infected MDCK cells showed a granular, focal fluorescence of the nucleus typical of Borna virus-infected cells that are exposed to virus-specific antibodies from infected rabbits and rats (Fig. 1). In no case did the antibody-containing serum of these patients react with uninfected MDCK cells.

Most of the patients with positive antibody titers had a cyclic form of affective disorder (that is, recurrent unipolar depression or bipolar affective disorder). Further clinical evaluation failed to reveal any other clinical features that distinguished the patients with Borna virus antibodies from patients who showed no such antibodies. It should be emphasized that the samples from the patients and the control subjects were randomly distributed and coded before they were analyzed.

There appear to be no reports on the presence of Borna virus in the United States even though the disease has been reported in horses in Europe for over 150 years. The virus isolated in Europe may not be identical with the strain now appearing in infected humans. Since the virus undergoes variation while adapting to growth in a given species (*10*), a variant strain may be responsible for the human infection.

The finding that more than 4 percent of the patients in Philadelphia were positive for Borna virus antibodies whereas less than 1 percent of the patients in Giessen and Würzburg were positive might be explained by the more heterogeneous nature of the patient population from Germany. Although one cannot draw a parallel between human psychiatric disorders and disorders of animal behavior, it is interesting that in at least two species of animals, the tree shrew and laboratory rat, behavioral disorders have been reported as one of the major manifestations of infection with Borna virus (*5*, *6*). Our data suggest that there may be a specific relation between exposure to Borna virus and development of an affective disorder, particularly of a cyclic nature. Further studies are needed to evaluate this hypothesis.

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