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Tardive dyskinetic syndrome in rats infected with Borna disease virus

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Summary Tardive Dyskinesia (TD) is a hyperkinetic movement disorder caused by chronic treatment of psychiatric patients with dopamine (DA) receptor blocking drugs (Stacy & Jankovic 1991). Although TD is one of the most important and frequently encountered iatrogenic disorders in clinical medicine, its pathophysiology is poorly understood. We have observed a hyperkinetic movement disorder in rats experimentally infected with a neurotropic RNA virus, Borna disease virus, that may provide important insights into the pathophysiology of TD. Like TD patients, infected rats show prominent orofacial dyskinesias. In keeping with the dopamine (Goetz & Klawans 1982) and anatomic (Fibiger & Lloyd 1984) hypotheses of TD, the Borna disease rat model shows enhanced behavioural sensitivity to DA agonists and selective striatal cell damage. There is also evidence of DA deafferentation and heterogeneous reduction of D2 binding in the caudate-putamen, particularly from sites implicated in oral behaviour. These observations on a virus-induced movement disorder offer novel approaches to TD pathogenesis.

Keywords animal model, Borna disease virus, dopamine, dyskinesia

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Introduction

Borna disease virus (BDV) is an RNA virus that causes a progressive central nervous system (CNS) disorder in immunocompetent animals (Borna disease, BD) (Ludwig *et al.* 1988). The virus has worldwide distribution and naturally infects a wide variety of vertebrate hosts including horses, sheep, cattle, cats and domestic fowl (Lipkin & Koprowski 1995). Primates, such as tree shrews and rhesus monkeys, are susceptible to experimental disease (Sprankel *et al.* 1978, Stitz *et al.* 1980). An increased prevalence of antibodies reactive with BDV proteins has been reported in subjects with neuropsychiatric disorders, suggesting the possibility that BDV or a related virus may cause human disease (Rott *et al.* 1985, Fu *et al.* 1993).

BDV is a novel infectious agent. The negative-sense RNA genome was recently cloned to reveal only limited similarities in sequence and organization to other viruses (Briese *et al.* 1994, Cubitt *et al.* 1994). *In vivo* and *in vitro*, BDV establishes persistent neuronal and glial infections. Important as a virus with unique biology and as a potential human pathogen, BDV can now be linked to another condition of monoamine transmitter imbalance, a dyskinetic movement disorder.

Methods

Infection of animals

Under metofane anaesthesia, 1-month-old male Lewis rats (Charles River Labs, Wilmington, MA, USA) were inoculated intracerebrally (i.c.) into right lateral ventricle with either 1.6×10^4 tissue culture infectious dose units of BDV (BD rats), or phosphate buffered saline (PBS, normal control, NL rats) in a total volume of 30 μL. Virus stock was a 10% wt/vol BD rat brain homogenate in PBS (Carbone *et al.* 1987). Infection was confirmed by the appearance of a clinical syndrome consistent with BD and the presence of antibodies reactive with viral proteins by Western blot. Animals were tested or killed 45 days after i.c. inoculation with either virus or PBS.

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Drugs

Drugs used were: d-amphetamine sulfate (indirect DA agonist) (Smith, Kline and French, Philadelphia, PA, USA) at doses of 0, 0.25, 0.50 and 1.00 mg kg^{-1} subcutaneously (s.c.); apomorphine HCl (direct DA agonist) (Sigma, St Louis, MO, USA) at doses of 0, 25, 50 and 100 μg kg^{-1} s.c. and 0, 0.25, 0.50 and 1.00 mg kg^{-1} s.c.; raclopride (D2 antagonist) (Astra, Sodertalje, Sweden) at doses of 0, 25, 50 and 100 μg kg^{-1} s.c.; SCH23390 (D1 antagonist) (Schering, Union, NJ, USA) at does of 0, 5, 10 and 20 μg kg^{-1} s.c.; or Clozapine (atypical neuroleptic) (Sandoz, East Hanover, NJ, USA) at doses of 0, 3, 6 and 12 mg kg^{-1} intraperitoneally. Clozapine was dissolved in a minimal amount 1 N HCl and diluted with distilled water. All other drugs were dissolved in normal saline. One of three doses of test drug or its vehicle control was given to each animal according to a Latin square design to control for conditioning and order effects. Each animal received one drug.

Behaviour analysis

Behaviour was monitored continually in 40×25×20 cm cages equipped with two equally spaced horizontal photocell beams across the long axis. Locomotor activity was quantified by numbers of crossovers (the successive interruption of the two photobeams). Stereotypical behaviour was scored through direct observation (MacLennan & Maier 1983) by an experienced observer who was blind to drug dose. Photocell beam interruptions and crossovers were analysed by using a repeated measures analysis of variance (ANOVA) design: infected or non-infected groups formed the independent factor, dose and time were the repeated measures. Subsequent group effects were analysed with simple main effects (Winner *et al.* 1991). Individual means comparisons were analysed using *post hoc* Newman-Keuls tests. Significance was taken at $P<0.05$. Observational data were analysed using the Information Statistic (Kullback 1968).

Neurochemistry

Six BD rats and eight NL rats were killed. After decapitation, brains were removed and dissected over ice. A 2-mm thick coronal section was taken between 1 and 3 mm posterior to the anterior genu of the corpus callosum. The caudate-putamen (CP), nucleus accumbens (NA) and olfactory tubercle (OT) were dissected from the coronal section. All tissue pieces were frozen immediately using dry ice. Tissue levels of dopamine and DOPAC were determined using high-pressure liquid chromatography (HPLC) with electrochemical detection (Jakubovic *et al.* 1987). Tissues were homogenized for 30 s in 0.2 M $HClO_4$ containing 0.15% $Na_2S_2O_5$, 0.5% Na_2 EDTA and 1 μM isoproterenol (internal standard). Following centrifugation at 14000 rpm for 15 min at 4°C, the supernatant was removed and filtered with 0.2 μm Nylon-66 membrane filters (Whatman, Hillsboro, OR, USA) and stored at −80°C. The HPLC system consisted of a Rainin HPXL solvent Delivery System, Macintosh with Dynamax Software, ESA Coulochem Model 5100A Electrochemical detector with Model 5011 High Sensitivity Analytical Cell, and Beckman Ultrasphere C18 column (3 μm, 75×4.6 mm). The detector potential was set at +0.45 V, sensitivity 10 nA/V. The mobile phase consisted of 100 mM sodium phosphate (monobasic) (pH 3.0), with 0.1 mM EDTA, 1.0 mM heptane sulfonic acid, 5.0% acetonitrile, 0.01% triethylamine. The detection limit of the system was approximately 0.4 pmol 100 μL^{-1} of tissue sample.

Neuroanatomy

Under metofane anaesthesia, BD rats and NL rats were perfused with buffered 4% paraformaldehyde. Next, brains were removed, postfixed overnight then cryoprotected using 19% sucrose in PBS. Twenty-micron coronal sections through olfactory bulb, prefrontal cortex, anterior cingulate and CP, thalamus, midbrain, pons and medulla were collected on to chrom/alum coated slides for immunohistochemistry or *in situ* hybridization. Methods for *in situ* hybridization to BD rat brain have been described (Carbone *et al.* 1991). Sections were hybridized with a [^{35}S]RNA probe complementary to mRNA encoding a 23 kDa protein of BDV, incubated with RNase to reduce non-specific hybridization, washed and dehydrated for autoradiography against Cronex film (Dupont, Wilmington, DL, USA). Sections were then dipped in NTB2 emulsion (Kodak, Rochester, NY, USA) and stained with cresyl violet for microscopic analysis. For immunohistochemistry, sections were incubated first with rabbit antibodies to tyrosine hydroxylase (TH) (Eugene Tech, Allendale, NJ, USA) and then with an avidin-biotin-peroxidase complex (ABC) system (Vector Labs, Burlingame, CA, USA). TH-positive cells were counted using 100-μm microscope eyepiece grids.

Quantitative receptor autoradiography

Metofane anaesthetized BD rats and NL rats were perfused with cold PBS followed by 0.1% paraformaldehyde to limit infectivity and maintain tissue integrity. D1 receptors were detected by incubating slide-mounted tissue sections with 0.5–8.0 nM [^{3}H]SCH 23390 (New England Nuclear, Boston, MA, USA) in the presence of 1 μM mianserin (Sigma); D2 receptors with 0.3–0.4 nM [^{3}H]raclopride (New England Nuclear). Non-specific D1 binding was defined by incubating adjacent sections with 1 μM SKF83566 (Research Biochemicals International, Natick, MA, USA), non-specific D2 binding with 1 μM (+)-butaclamol (Research Biochemicals International) (Lidow *et al.* 1991). Tissue sections from BD and NL animals were processed simultaneously. Autoradiograms were analysed with

a computer-based image analysis system (MCID, Imaging Research Inc., St Catherine, Ontario, Canada). Measurements were taken across coronal sections of CP and its functional subregions: dorsolateral (limb movement and somatosensory orientation: Joyce & van Hartesveldt 1984, Fairley & Marshall, 1986), ventrolateral (oral behaviour, Kelley *et al.* 1988), medial (conditioned reinforcement, Kelley & Delfs 1991). Calibration curves were constructed using [^{3}H]polymer standards (Amersham, Arlington Heights, IL, USA). Analysis of saturation binding, based on 10 different concentrations of each radioligand, utilized the non-linear curve fitting computer programs KINETIC, EBDA, LIGAND, LOWRY (Elsevier-BIOSOFT, Cambridge, UK). Scatchard plots generated by least square linear regression were used to estimate affinities (K_D) and concentration of receptor sites (B_{max}).

Results

Neuropharmacology

Forty-five days after infection, rats showed hyperactivity and spontaneous dyskinesias (vacuous chewing, mouth opening, head bobbing, upper body tics), retrocollis, dystonias and flexed seated postures, suggesting a syndrome of DA sensitivity. The pharmacology of the syndrome was initially investigated using dopaminergic drugs. BD rats showed enhanced behavioural sensitivity to DA agonists and to DA antagonists with D1 activity, compared with normal controls.

BD rats had increased sensitivity to the indirect DA agonist, d-amphetamine, manifested by increased amphetamine-induced locomotion and stereotypes. For d-amphetamine induced locomotor activity (Fig. 1a), individual means comparisons revealed a significant increase in locomotor activity in the BD group at the 0.5 mg kg^{-1} dose compared with saline. This dose was also significantly different from the NL rats (Table 1). For d-amphetamine stereotyped behaviour (Fig. 1b), there was a significant increase in stereotyped behaviour ratings in the BD rats. Subsequent examination at each dose level revealed significant increases at doses of 0.25, 0.50 and 1.00 mg kg^{-1} (Table 1).

The direct DA agonist, apomorphine, at low doses, had an enhanced sedative effect, indicating an increased sensitivity of BD rats to presynaptic (autoreceptor) doses of the drug. For the locomotor response to apomorphine (Fig. 1c), individual means comparisons revealed a significant decrease in locomotor activity at all doses of apomorphine (Table 1). Apomorphine, at higher doses, caused a dose-dependent increase in locomotor activity in controls and a dose-dependent decrease in locomotor activity in BD rats, with BD rats showing more stereotyped behaviour.

The atypical neuroleptic, clozapine, which has D1 and D2 antagonist activity, selectively reduced activity in BD rats, but not in controls. For the locomotor response to clozapine (Fig. 1d), individual means comparisons revealed a significant decrease in locomotor activity at the two highest doses of clozapine (Table 1).

The selective D1 antagonist SCH23390 modified the locomotor response in the BD rats at doses that had no effect on the NL rats. Individual means comparisons revealed a significant increase in locomotor activity at the lowest dose of SCH23390 (5 μg kg^{-1}) and subsequent reversal of this effect at the higher doses (10 and 20 μg kg^{-1}) (Newman-Keuls test). The lowest dose of SCH23390 suppressed stereotyped behaviour, releasing a greater locomotor response in BD rats, while the higher doses caused sedation.

The D2 antagonist raclopride had no statistically significant effect on locomotor response.

Table 1. Statistically significant pharmacologic results. Significance was determined by ANOVA for crossover data and by InfoStat for observational data (Î=information statistic)

ANOVA		
d-amphetamine	Group×dose	$F=3.873$, df=3,18, $P<0.05$
	BD dose main effect	$F=8.935$, df=3,18, $P<0.05$
	0.5 mg kg^{-1} BD vs. NL main effect	$F=5.397$, df=1,13, $P<0.05$
apomorphine	Group×dose	$F=4.922$, df=3,18, $P<0.05$
	BD dose main effect	$F=12.70$, df=3,18, $P<0.05$
clozapine	Group×dose	$F=2.750$, df=3,18, $P=0.07$
	Group×dose (log)	$F=6.550$, df=3,18, $P=0.003$
	BD dose main effect	$F=4.090$, df=3,18, $P<0.05$
SCH23390	Group×dose	$F=3.947$, df=3,18, $P<0.05$
InfoStat		
d-amphetamine	Overall	$2\hat{I}=29.8$, df=4, $P<0.05$
	0.25 mg kg^{-1}	$2\hat{I}=6.09$, df=1, $P<0.05$
	0.50 mg kg^{-1}	$2\hat{I}=11.09$, df=1, $P<0.05$
	1.00 mg kg^{-1}	$2\hat{I}=11.09$, df=1, $P<0.05$

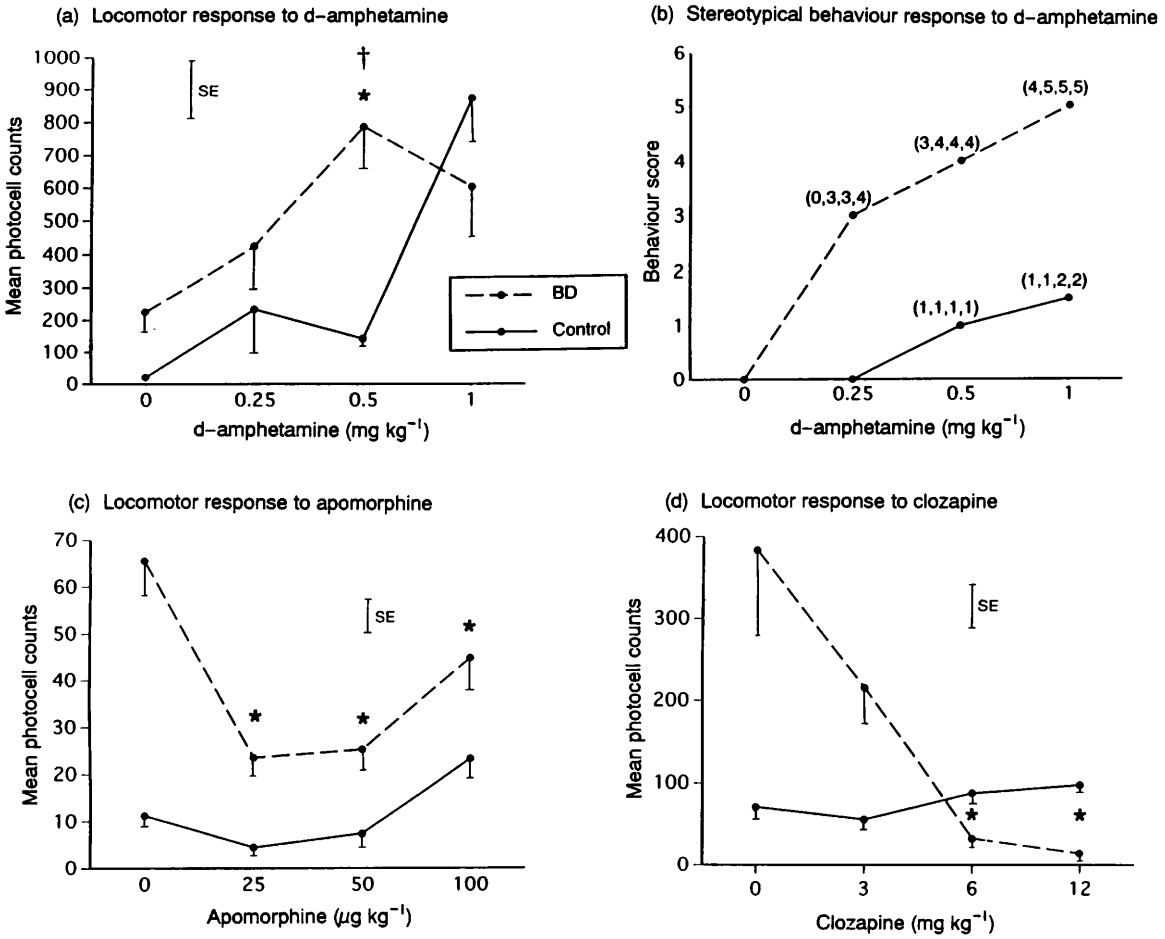

Fig. 1. Locomotor and behaviour responses to DA agonists and antagonists in BD and noninfected (NL) rats. (a) Locomotor response to d-amphetamine in BD and NL rats (n=4 each group). Values represent mean locomotor counts over 180 min. SE=average SEM (Rassnick *et al.* 1993). * Indicates significant increase in locomotor activity in BD rats at this dose relative to saline injection, Newman-Keuls test following significant ANOVA BD dose main effect. † Indicates significant increase in locomotor activity in BD group versus control group, 0.5 mg kg−1 BD vs. control main effect following significant 2 factor (group×dose) ANOVA. (b) Stereotypical behaviour response to d-amphetamine in BD and NL rats. Values represent median score using the MacLennan and Maier scale (MacLennan & Maier 1983). BD rats typically had scores of 3 (0, 3, 3, 4) at the low dose, 4 (3, 4, 4, 4) at the 0.5 mg kg−1 dose, and 5 (4, 5, 5, 5) at the highest dose. Stereotypical behaviour ratings were significantly increased in BD rats at these doses. Information Statistic revealed a significant increase in stereotyped behaviour ratings in the BD rats; subsequent examination at each dose revealed significant increases at doses 0.25, 0.50 and 1.00 mg kg^{-1}. (c) Locomotor response to low-dose apomorphine in BD and NL rats (n=4 each group). Values represent mean over 15 min. SE=average SEM. * Indicates significant decrease in locomotor activity in BD rats at these doses relative to saline injection, Newman-Keuls test following significant ANOVA BD dose main effect. (d) Locomotor response to clozapine in BD and NL rats (n=4 each group). Values represent means over 180 min. SE=average SEM. * Indicates significant decrease in locomotor activity in BD rats at these doses relative to pH balanced dH_2O injection, Newman-Keuls test following significant ANOVA BD dose main effect.

Neuroanotomy

In situ hybridization analysis was performed to determine the distribution of viral nucleic acids in BD rat brain. Viral RNA was found throughout limbic circuits, basal ganglia structures, layers II, III, V and VI of neocortex and allocortex and monoamine cell groups (Fig. 2). Highest levels of viral RNA were in the prefrontal system (DA neurones of VTA, amygdala, anteromedial CP, and prefrontal/orbital/anterior cingulate cortices) mesolimbic system (VTA, shell region of NA, ventral striatum), nigrostriatal system [DA neurones of substantia nigra pars compacta (SNc) and their projections to dorsal CP], in locus coeruleus, and, to a lesser extent in raphe nuclei. No virus-specific hybridization signal was found in NL rat brain (data not shown).

Neurochemistry

The neurochemistry of the syndrome was investigated by measuring tissue levels of DA and its primary metabolite

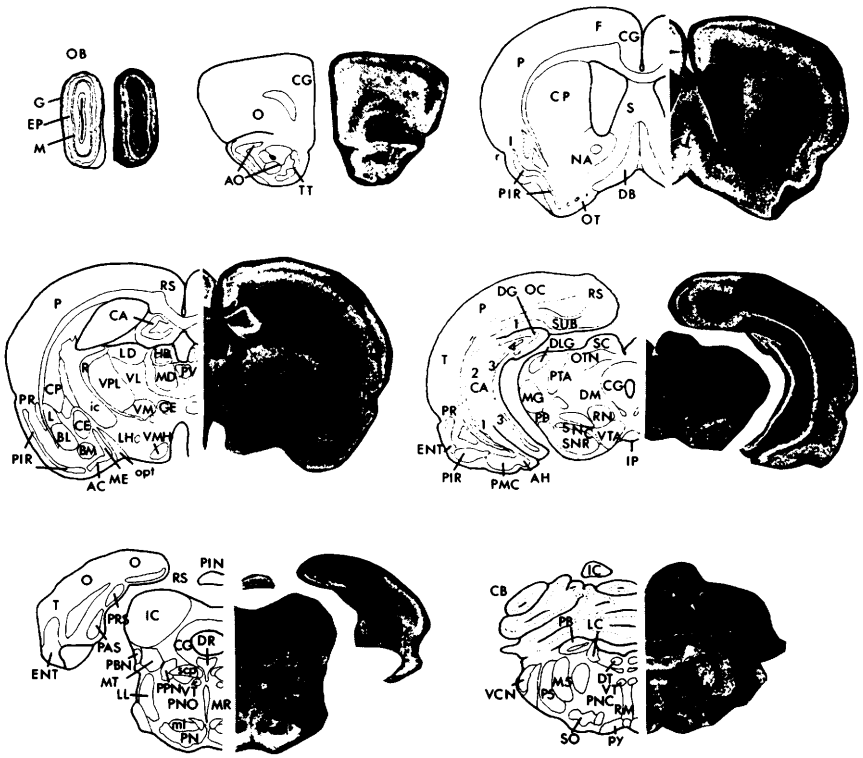

Fig. 2. Borna disease virus (BDV) nucleic acids in coronal sections of infected animals, 20-μM sections through olfactory bulb, prefrontal cortex, anterior cingulate and caudate-putamen, thalamus, midbrain, pions and medulla of adult Lewis rat 45 days post-i. c. inoculation with BDV were hybridized with an [^{35}S]RNA probe complementary to an mRNA encoding the 23 kD protein of BDV. Abbreviations: CG — cingulate cortex, O — orbital cortex, F — frontal motor cortex, P — parietal sensorimotor cortex, PIR — pinform cortex, OT — olfactory tubercle, DB — diagonal band, CP — caudate-putamen, NA — nucleus accumbens, RS — retrosplenial cortex, CA — hippocampal formation, MD — mediodorsal nucleus (thal), Amygdala Complex nuclei (CE — central, BL — basolateral, L — lateral), SNc — substantia nigra pars compacta, VTA — ventral tegmental area, MR — median raphe, LC — locus coeruleus.

DOPAC by HPLC from CP, NA and OT of NL and BD rats (Table 2). Infection resulted in DA depletions of 47.3% in CP, 29.4% in NA and 30.4% in OT, with significant effect on DA levels in CP F=4, df 1, 12, $P<0.05$.

Numbers of dopaminergic cells were assessed by TH-immunohistochemistry. TH-positive cells were significantly reduced in SNc 45 days after i.c. infection. The average numbers of TH-positive cells per hemisphere per slide for three animals ± SEM in NL and BD groups were: (NL 158.33 ± 16.51, BD 87. ± 673.48) (*t*-test, $P<0.05$) in SNc, and (NL 191.00±10.82, BD 174.67 ± 2.03) in VTA.

Receptor autoradiography

Post-synaptic changes in DA system were examined by quantitative receptor autoradiography. Figure 3 shows labelling in CP of NL and BD rats of D2 sites by raclopride and D1 sites by SCH23390. In BD rats, specific binding of 1 nM [^{3}H]raclopride, expressed as fmol mg–l tissue, was significantly decreased in CP and each of its four subregions (Table 2). Specific binding of [^{3}H]SCH23390 in CP of BDV-infected rats was unchanged from control values (Table 3). Saturation studies were based on 10 concentrations of each radioligand. K_D and B_{max} values of CP D2 receptors obtained in NL rats were 1.002 nM and 154.20fmol mg protein^{-1}; in BD rats were 1.213 nM and 90.93fmol mg protein^{-1}. KD and B_{max} values of D1 receptors in CP of NL rats were 1.430 nM and 445.9fmol mg protein^{-1}; in BD rats were 1.171 nM and 399.48 fmol mg-protein^{-1}.

Discussion

We have established a new model for addressing the pathophysiology of dyskinetic disorders, based on experimental infection with BDV. Previous descriptions of disease in rats infected with either strain V or He/80 of BDV distinguished a transient encephalitic aggressive phase from a chronic apathetic phase with premature senescence (Narayan *et al.* 1983). Although abnormalities in mood and intellect may be present, we have been more impressed by the dramatic extrapyramidal syndrome of BD. This paper describes and characterizes a previously unrecognized intermediate stage of BD in the rat, which is apparent 6 weeks

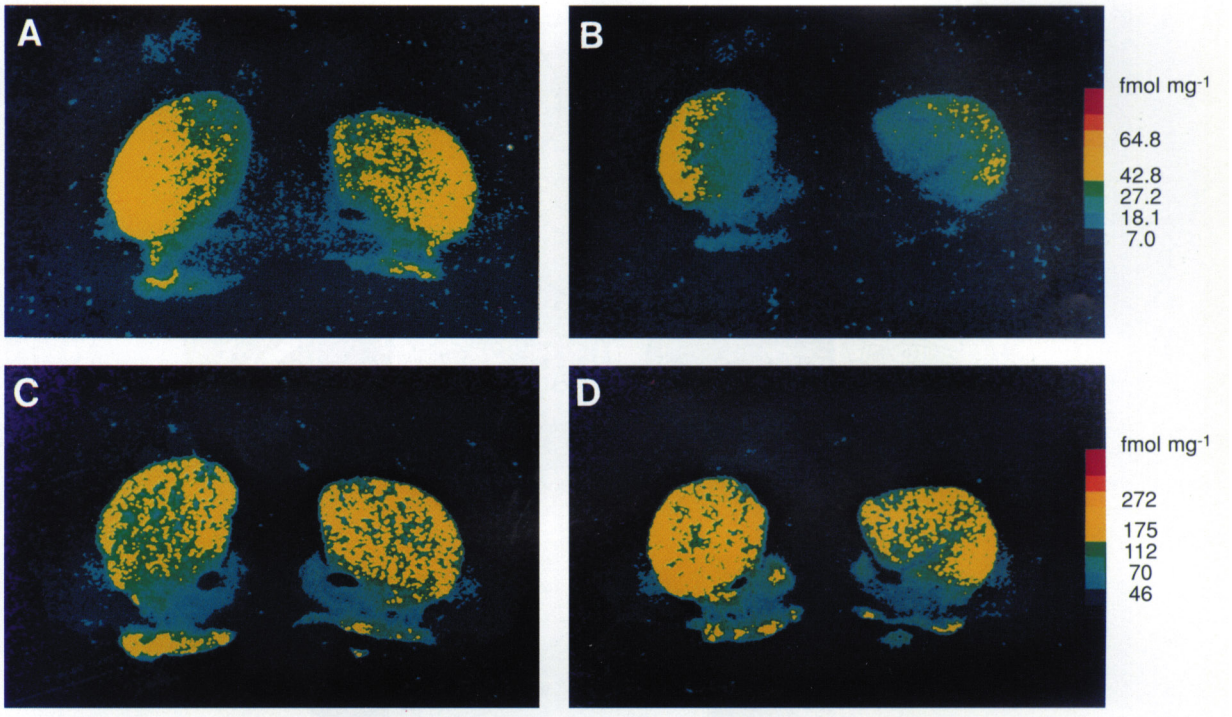

Fig. 3. DA site labelling in coronal sections through caudate-putamen (CP) rostral to the decussation of the anterior commissure in normal (NL) and infected (BI) rats 45 days post-i.c. injection of either PBS or BDV D2 site labelling by (1 nM) [^{3}H]raclopride in NL (A) or BD (B) rats Specific binding of [^{3}H]raclopride was significantly decreased in CP and each of its four subregions in BD rats DI site labelling by (2 nM) [^{3}H]SCH23390 m NL (C) or BD rats (D) Specific binding of [^{3}H]SCH23390 in CP of BD rats was unchanged from control values.

after i.c. infection and stable for the next 3 months. The application of a neural systems approach to analysis of this syndrome led to the discovery of specific disturbances in nigrostriatal and mesolimbre DA systems as well as pathological changes in the CP.

BD rats had spontaneous dyskinesias that appeared similar to the spectrum of orofacial and generalized choreic and dystonic movements of TD (Stacy & Jankovic 1991). Pharmacological responses to DA agonists and antagonists, shown in Fig. 1, suggest that BD, like TD, is a syndrome of increased DA sensitivity. For example, the BD dyskinetic syndrome is aggravated by the indirect DA agonist d-amphetamine and improved by the atypical neuroleptic clozapine which has D1 and D2 DA antagonist activity.

In BD rat brain, viral RNA was found in non-homogeneous distribution throughout limbic and catecholamine circuits (Fig. 2). Multisystem atrophy was correlated with virus distribution with an estimated 20–40% loss of volume from prefrontal cortex, periventricular, limbic, basal ganglia and mesencephalic structures. Similar patterns of atrophy, particularly in regions that exercise extrapyramidal control of motor function, have been observed in TD patients. Enlarged third and lateral ventricles, accompanied by striatal atrophy, are identified risk factors for TD among schizophrenic patients receiving conventional neuroleptics (Bartels & Themehs 1983).

To test the hypothesis that the substrate for TD-like syndrome is an abnormal C.P. we examined the effects of infection on striatal DA pre- and post-synaptic elements. Tissue levels of DA and its primary metabolite DOPAC were measured by HPLC. In BD rats, DA depletions ranged from 30% in NA and OI to 47% in CP, with significant effect on DA levels in CP (Table 1) The decreases in DOPAC levels in CP and limbic areas were less than the changes in DA concentration, suggesting partial DA denervation and compensation within residual DA terminals. Partial lesions in nigrostriatal and mesolimbic DA circuits were confirmed by loss of TH-immunoreactive cells from the SNc and VTA Partial DA denervation, like DA receptor blockade by neuroleptics, would deprive striatal targets of usual levels of DA stimulation, creating a condition that may be functionally equivalent to chronic treatment with DA receptor antagonists. Alternatively, partial DA cell loss in BD rat may create a neuropathological condition analogous to subclinical Parkinsonism, itself a risk factor for TD. An early and severe Parkinsonian reaction to neuroleptics is one of the better predictors of later development of TD (Miller & Choumard 1993)

To analyse a post-synaptic effect, we examined D1 and D2 receptors in striatal sections by quantitative autoradiography (Fig. 3) BD rats and NL rats showed no difference in D1 receptor binding in CP. In contrast, BD rats had a

Table 2. Levels of dopamine and DOPAC in caudate-putamen (CP), nucleus accumbens (NA) and olfactory tubercle (OT) of Noninfected (NL) and Borna disease virus-infected (BD) rats

Site	Dopamine	DOPAC	DOPAC/dopamime
CP			
NL (n=8)	7.04±0.91	2.07±0.20	0.32±0.05
BD (n=6)	3.71±0.53*	1.41±0.22*	0.39±0.04
Depletion	47.3%	31.9%	
NA			
NL (n=6)	3.98±0.92	1.39±0.30	0.36±0.02
BD (n=6)	2.81±0.43	1.06±0.11	0.41±0.06
Depletion	29.4%	23.4%	
OT			
NL (n=7)	2.66±0.43	0.927±0.13	0.41±0.11
BD (n=6)	1.85±0.57	0.891±0.25	0.67±0.17
Depletion	30.4%	3.9%	

Dopamine and DOPAC values in micrograms per gram wet weight of tissue, mean±SEM.
*P<0.05 (two-tailed t-test), significantly different from normal rat group.

significant decrease in D2 receptor binding in CP and each of its four subregions, especially the two medial subregions, areas that receive input from locations of earliest infection (basolateral nucleus of amygdala, VTA, and prefrontal cortex: Solbrig *et al.* 1995) and the ventrolateral region, a site specific for oral behaviour (Kelley *et al.* 1988). The reduction in [^{3}H]raclopride binding may correlate with the pharmacological insensitivity of BDV rats to raclopride, noted earlier. Saturation analysis indicated a 41% reduction in the number of D2 receptors with no change in affinity. Mazindol binding to high-affinity DA uptake sites, a pre-synaptic marker of DA terminal density (measured using the method of Smith *et al.* 1993) was reduced by only 12% (data not shown), indicating that reduced numbers of D2 receptors could not be attributed to loss of pre-synaptic D2 receptors. Reduced numbers of D2 receptors could not be attributed to volume changes, either. While differences in brain volume may result in different density values for receptors, the brains of BD rats were consistently smaller than those of NL rats, a volume change which would obscure a significant reduction in D2 receptor numbers. Decreased acetylcholinesterase staining in the striatum (data not shown), where a proportion of striatal cholinergic cells express D2 receptors (Weiner *et al.* 1990), together with the mazindol data, supports a post-synaptic site for D2 receptor reduction. Since the great majority of cells in the striatum are GABAergic medium spiny neurones (Ribak *et al.* 1979), many of the D2-expressing neurones damaged by BDV may be GABAergic. Our model is consistent with both an anatomical GABA hypothesis for TD, that neuroleptic-induced damage to subpopulations of striatal GABA-containing neurones causes TD (Fibiger & Lloyd 1984), and

Table 3. Specific binding of dopamine receptor ligands [^{3}H]raclopride and [^{3}H]SCH23390 to caudate-putamen and each of its four subregions in normal (NL) and Borna disease virus-infected (BD) rats

	(1 nM) [^{3}H]raclopride		(2 nM) [^{3}H]SCH23390	
Site	NL (n=3)	BD (n=3)	NL (n=3)	BD (n=3)
CP	47.94±0.87	41.18±0.65**	180.35±3.85	168.40±4.43
DL	57.28±2.47	49.14±1.62*		
DM	47.51±1.44	35.80±1.50**		
VL	60.47±4.84	42.20±3.33**		
VM	39.33±0.81	24.70±0.93**		

Specific binding values in fmol mg^{-1} of wet weight of tissue, mean±SEM.
*P<0.05, **P<0.01 (t-tests), significantly different from normal rat group.
Caudate putamen, CP; subregions of CP; dorsolateral, DL; dorsomedial, DM; Ventrolateral, VL; ventromedial, VM.

the experimental finding that post mortem human brain homogenates from TD patients have diminished D2 receptor density in striatal regions (Reynolds *et al.* 1992).

We suggest that the pathological changes which give rise to dyskinesias of the BD rat have both pre- and post-synaptic components. In the BD rat, the projection fields of surviving DA neurones may widen after partial DA deafferentation, mixing inputs from normally parallel, functionally segregated basal ganglia circuits. For example, cortical signals from orofacial motor areas in ventrolateral CP (Ebrahimi *et al.* 1992) may overlap amygdalostriate signals in contiguous ventral areas (Kelley *et al.* 1982). The degree of overlap predicts the presence and nature of dyskinetic symptoms if overlap occurs in areas where D2 receptors or D2-expressing neurones are lost. Pathological changes in the indirect striatal outflow pathway, comprised of D2-expressing neurones (Gerfen *et al.* 1990), have already been linked to such dyskinetic syndromes as the chorea of Huntington's (Reiner *et al.* 1988) and hemiballismus following subthalamic nucleus lesions (DeLong 1990). In BD, vulnerable striatal regions were target areas of early–infected limbic and pre-frontal pathways.

The movement disorder in BD rats is similar in phenotype, neuropharmacology, and neurochemistry to neuroleptic-induced TD. Borna disease, an experiment of nature, causing CNS cell loss and abnormal sensitization, has an additional interesting parallel to neuroleptic toxicity. Just as long-term exposure to neuroleptics may not be limited to an extrapyramidal syndrome: widespread atrophy of retinal pigment epithelium and photoreceptor cells is another side effect of extended neuroleptic use (Green 1985); chronic BDV infection causes not only a movement disorder, but also a retinopathy leading to blindness. Chronically infected BD rats have high levels of virus in the retina (Narayan *et al.* 1983) and bleaching of this structure progresses for months after inflammation has resolved. Though the mechanisms of BDV tropism and selective cytopathic effect are not known, it is intriguing that D2 receptors and rhodopsin are related by membership in the G-protein coupled superfamily of receptors (Findlay *et al.* 1993). While a single neurochemical hypothesis of TD is unrealistic, a model based on persistent CNS viral infection and its effect on signal transduction departs from ligand-defined receptor subtype models and provides a new approach to the problem of TD pathogenesis.

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