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A Neural Substrate of Hyperactivity in Borna Disease: Changes in Brain Dopamine Receptors

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Rats experimentally infected with the neurotropic RNA virus, Borna disease virus, have a hyperactive movement disorder. Because locomotor activity is modulated by the nucleus accumbens (N. Acc.) dopamine (DA) system, high-affinity DA uptake, DA D1, D2, and D3 receptor binding sites were examined in N. Acc. subregions of normal and infected rats by quantitative receptor autoradiography. The N. Acc. of infected rats had decreased mazindol and D2 and D3 radioligand binding in the core and decreased D3 radioligand binding in rostral subregions. The abnormalities observed in the N. Acc. DA system of infected rats may offer insights into the potential viral pathogenesis of psychiatric conditions with a dopaminergic substrate such as schizophrenia and affective disorders.

INTRODUCTION

Borna disease virus (BDV) is a neurotropic negative-strand RNA virus that causes disturbances of movement and behavior in a wide range of animal species (Narayan *et al.,* 1983; Ludwig *et al.,* 1988; Solbrig *et al.,* 1994). The virus is a natural pathogen of several domestic mammal and bird species in Europe and the Middle East (Koprowski and Lipkin, 1995) and is serologically linked to psychiatric syndromes including schizophrenia and affective disorders (Rott *et al.,* 1985; Fu *et al.,* 1993; Bode *et al.,* 1995; Waltrip *et al.,* 1995). In experimental and natural disease, BDV nucleic acids and proteins are found in limbic (Carbone *et al.,* 1987; Lipkin *et al.,* 1990) pyramidal, and extrapyramidal motor circuits (Solbrig *et al.,* 1994).

A primary site of integration between limbic and motor information is a ventral striatal structure, the nucleus accumbens (N. Acc.) (Mogenson *et al.,* 1980). Experimental evidence suggests that the N. Acc. is important in goal-oriented behaviors, locomotor activation, response to novel stimuli, and the reinforcing properties of psychostimulant drugs (Swerdlow *et al.,* 1986; Le Moal and Simon, 1991; Koob, 1992). The dopaminergic mesoaccumbens–pallidal circuit, implicated in the control of motor, cognitive, emotional, and reward processes, may be important to the pathogenesis and treatment of schizophrenia, depression, and human drug-seeking behaviors.

The advent of experimental models of Borna disease (BD) in rats and widened knowledge of neuropharmacology in the mesoaccumbens–pallidal circuit affords opportunities to use neurotransmitter-specific behavioral paradigms to examine viral effects. In fact, the hyperactivity observed in BD rats is similar to that found after experimental manipulations of the rat mesolimbic DA system. It is well established that the direct DA agonist, apomorphine, induces a profound hyperactivity response in rats with a 6-hydroxydopamine (6-OHDA)-induced denervation of N. Acc. (Kelly and Iversen, 1975; Van der Kooy *et al.,* 1983). Given the importance of N. Acc. DA in motivational, appetitive, and locomotor behaviors, the current study was initiated to determine whether the abnormal behavior seen in the BD rat could be correlated with established pharmacologic models of locomotor hyperactivity due to activation of N. Acc. DA receptors. To address this hypothesis we performed a quantitative anatomic analysis of DA high-affinity uptake sites and D1, D2, and D3 receptor binding in N. Acc. of normal and BD rats.

MATERIALS AND METHODS

Infection of animals

Under metofane anesthesia, twenty-five 1-month-old male Lewis rats (Charles River Labs) were inoculated intracerebrally (ic) into the 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% w/v 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 used for behavioral analysis or sacrificed 45 days after ic inoculation.

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Behavior analysis

Behavior was monitored continually in 40 × 25 × 20-cm cages with two equally spaced horizontal beams across the long axis. Locomotor activity was quantified by numbers of crossovers (the successive interruption of the two photobeams). Locomotor activity was recorded every 30 min for 180 min. Photocell beam interruptions and crossovers were analyzed by using a repeated-measures analysis of variance (ANOVA) design: infected or noninfected groups formed the independent factor and time was the repeated measure. Subsequent group effects were analyzed with simple main effects (Winer *et al.,* 1991). Significance was taken at $P < 0.05$. Each experimental group contained eight animals.

Quantitative receptor autoradiography (AR)

BD rats and NL rats were anesthetized with methoxyflurane and perfused with cold PBS followed by 0.1% paraformaldehyde to limit infectivity and maintain tissue integrity (Lidow *et al.,* 1991). Comparable specific binding for 0.1% paraformaldehyde-fixed and unfixed tissue was verified for each radioligand.

Twenty-micrometer coronal sections were collected onto gelatin-subbed slides through the N. Acc. according to atlas coordinates of Paxinos and Watson (1986): at its rostral pole, Bregma +2.70 mm, and at the genu of the corpus callosum, Bregma +1.60 mm, where core and shell regions could be distinguished.

DA uptake sites were detected by incubating slide-mounted tissue sections with 8.0 n*M* [^{3}H]mazindol (New England Nuclear) in the presence of 300 n*M* desipramine (Sigma). Nonspecific binding was defined by incubating adjacent sections with 30 μM benztropine (Smith *et al.,* 1993). D1 receptors were detected by incubating sections with 2 n*M* [^{3}H]SCH23390 (New England Nuclear) in the presence of 1 μM mianserin (Sigma). Nonspecific D1 binding was defined by incubating adjacent sections with 1 μM SKF83566 (Research Biochemical International) (Lidow *et al.,* 1991). D2 receptors were labeled by 0.7 n*M* [^{3}H]spiperone (Amersham) in the presence of 40 n*M* ketanserin (Research Biochemical International). Nonspecific binding was defined by incubating adjacent sections with 1 μM (+)-butaclamol (Research Biochemical International) (Joyce *et al.,* 1985). D3 receptors were labeled by 0.2 n*M* ^{125}I-(*R*)-*trans*-7-OH-PIPAT (prepared by Dr. Mei Ping Kung, Department of Radiology, University of Pennsylvania School of Medicine) in the presence of 0.3 m*M* GTP and 5 μM DTG (to block sigma receptors). Nonspecific binding was defined by incubating adjacent sections with 10 μM 7-OH-DPAT (Kung *et al.,* 1994).

Tissue sections from BD and NL animals were processed simultaneously and then opposed to ^{3}H-sensitive film (Amersham). Autoradiograms were analyzed with a computer-based image analysis system (MCID, Imaging Research Inc.). Calibration curves were constructed using ^{3}H polymer standards (American Radiolabeled Corp.).

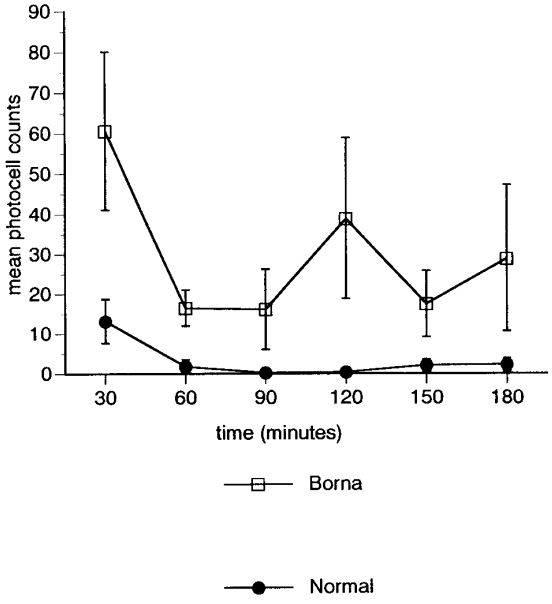

FIG. 1. Locomotor activity in normal rats and BD rats. ($n = 8$, each group). Values represent mean locomotor counts over 30-min intervals ± SEM.

After autoradiographic exposure, slides were stained with cresyl-violet for examination by light microscopy. Statistical significance between groups was determined by *t* test, with statistical significance set at $P < 0.05$.

Scatchard analysis of radioligand binding data could not be performed because of limited availability of the D3 ligand. Therefore, quantitative AR was performed at concentrations near the K_d of the D1, D2, and D3 radioligands where specific binding values plot along the linear phase of the saturation curves. For each radioligand, binding was repeated using a second radioligand concentration (0.4–0.5 times the K_d) to verify a consistent relation between normal and BD accumbens binding.

In situ hybridization

Under metofane anesthesia, 3 BD rats (45 days postintracerebral inoculation) and 3 NL rats were perfused with buffered 4% paraformaldehyde. Brains were removed, postfixed overnight, and then cryoprotected using 19% sucrose in PBS. Twenty-micrometer coronal sections were collected as described above. Sections from adult Lewis rats were hybridized with a ^{35}S-labeled RNA probe (specific activity 2×10^7 to 6×10^7 cpm/μg; 5 ng probe/slide) complementary to mRNA encoding the BDV P-protein. Detailed protocols for hybridization, autoradiography, and analysis have been described (Lipkin *et al.,* 1990; Carbone *et al.,* 1991).

RESULTS

Behavior

BDV produced a hyperactive syndrome 45 days after infection. Increased locomotor activity was continuous over the 180-min test sessions (Fig. 1). Two-way ANOVA

with repeated measures over time revealed a significant overall group difference in locomotor activity [Main effect: $F(1,14) = 7.018$, $P < 0.05$] and the time course of locomotor activity was significantly different between infected and control groups [Group $\times$ time interaction: $F(5,70) = 3.191$, $P < 0.05$].

In situ hybridization

In all BD rat brains analyzed, viral mRNA was detected in ventral striatal regions (Fig. 2): in the shell region of the N. Acc., in the olfactory tubercle, and, to a lesser extent, in core and rostral regions of N. Acc. No BDV-specific hybridization signal was found in NL rat brains (data not shown).

Quantitative receptor AR

BD rats showed reduced numbers of [^{3}H]mazindol-labeled DA uptake sites in all subregions of N. Acc. with significant reductions in the core (Table 1). Core regions in the N. Acc. of BD rats had a significant reduction in specific binding of [^{3}H]spiperone to D2 sites (compare Figs. 3C and 3D) and ^{125}I-(R)-*trans*-7-OH-PIPAT to D3 sites (compare Figs. 3G and 3H). In BD rats, D3 receptor binding was also reduced in rostral regions, overlapping the area where mazindol site reduction was close to significant (compare Figs. 3E and 3F). Specific binding of [^{3}H]spiperone to D2 sites was reduced in medial and ventrolateral caudate-putamen and in olfactory tubercle (compare Figs. 3C and 3D). Specific binding of [^{3}H]-SCH23390 to D1 sites in BD rats was unchanged from control values (Table 1).

DISCUSSION

The original clinical descriptions of natural BD included hyperactivity, excitability, and disorders of movement and posture (Zwick, 1939; Solbrig *et al.,* 1995). Experimental infection in rats led to recognition of BD as a multiphasic illness with an acute, aggressive phase, an intermediate phase characterized by hyperactivity and dyskinetic behaviors, and a chronic, docile, hypoactive phase characterized by premature senescence (Narayan *et al.,* 1983; Solbrig *et al.,* 1994). The hypoactive phase is associated with profound degeneration of efferent motor system components including motor neurons, corticospinal tracts, and cerebellum (Solbrig *et al.,* unpublished observations).

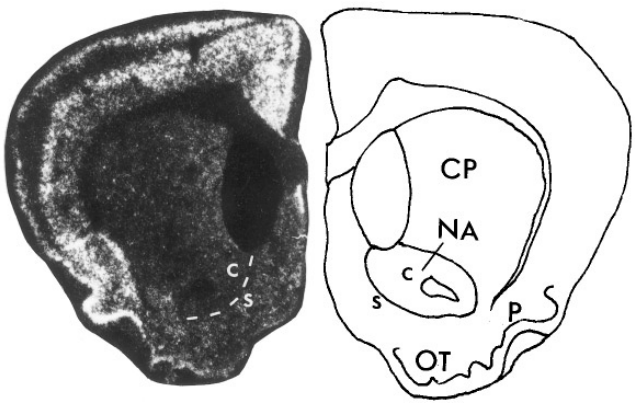

FIG. 2. Borna disease virus (BDV) nucleic acids in coronal section of adult Lewis rat 45 days following intracerebral infection. Twenty μm section through portion of nucleus accumbens where core and shell regions can be distinguished. Sections were hybridized with a ^{35}S-RNA probe complementary to mRNA encoding the P-protein of BDV. Dashed lines illustrate borders of core and shell. CP, caudate-putamen (striatum); NA, nucleus accumbens; s, shell subregion of NA; c, core region of NA; OT, olfactory tubercle; P, pyriform cortex.

TABLE 1

Specific Binding of [^{3}H]Mazindol, [^{3}H]SCH23390, [^{3}H]Spiperone, and ^{125}I-7-OH-PIPAT to Rostral, Core, and Shell Subregions of Nucleus Accumbens in Normal (NL) and Borna Disease Virus-Infected (BD) Rats

	Rostral	Core	Shell
Uptake sites (8 n*M* [^{3}H]mazindol)			
NL ($n = 3$)	470.14 ± 16.58	486.22 ± 31.57	252.32 ± 33.36
BD ($n = 5$)	364.38 ± 44.76	320.26 ± 47.07*	200.32 ± 32.52
D1 (2 n*M* [^{3}H]SCH23390)			
NL ($n = 5$)	1852.07 ± 8.40	1426.56 ± 90.42	1615.58 ± 95.32
BD ($n = 6$)	1816.11 ± 16.06	1236.34 ± 71.87	1505.55 ± 100.51
D2 (0.7 n*M* [^{3}H]spiperone)			
NL ($n = 5$)	192.31 ± 6.34	149.84 ± 9.60	102.77 ± 14.56
BD ($n = 5$)	194.01 ± 14.85	106.24 ± 7.24*	113.19 ± 10.33
D3 (0.2 n*M* ^{125}I-7-OH-PIPAT)			
NL ($n = 3$)	13.01 ± 0.37	15.66 ± 0.78	18.49 ± 0.98
BD ($n = 5$)	8.72 ± 0.44*	9.76 ± 1.12*	16.57 ± 0.45

Note. Specific binding values in fmol/mg protein, mean ± SEM.
* $P < 0.05$ (*t* test, significantly different from NL rat group).

In a previous report, dyskinesias in BD rats were linked to loss of the D2 striatal outflow pathway (Solbrig *et al.,* 1994). Although we found neurochemical evidence of augmented mesocortical DA activity, there were no changes in prefrontal cortex D1 or D2 receptors (Solbrig *et al.,* in press). Failing to find experimental evidence of partial DA denervation in prefrontal cortex to explain the observed hyperactivity, we examined the mesoaccumbens DA circuit.

Our findings indicate that the intermediate, hyperactive phase of BD in rats is accompanied by the loss of DA reuptake sites and selective decreases in DA receptor binding sites in rostral striatal areas, especially in the N. Acc. Pathology is more pronounced in core regions with a significant decrease in presynaptic reuptake sites as well as decreased numbers of D2 and D3 receptor binding sites. Given that BD rats have reduced levels of DA in the N. Acc. (Solbrig *et al.,* 1994), the presynaptic lesion, indicated by decreased mazindol binding, is likely to re-

flect DA terminal loss. While DA terminals have D3 receptors, D3 presynaptic autoreceptors represent only a minor proportion of D3 sites in N. Acc. (Diaz *et al.,* 1995). Alone, they are unlikely to account for the significant reductions in D3 binding observed in BD rats. Loss of DA innervation has been shown to lead to reduced postsynaptic D3 receptor expression (Levesque *et al.,* 1995). Thus, the decrease in D3 binding in core and rostral subregions of N. Acc., where mazindol binding is also reduced, may reflect reduced postsynpatic receptor expression after loss of DA or DA terminals.

The basis for decreases in D2 receptor binding in N. Acc. of BD rats is unclear. Midbrain 6-OHDA lesions usually result in striatal and N. Acc. D2 receptor upregulation (Creese *et al.,* 1977; Staunton *et al.,* 1982). In BD rats, the lack of a D2 receptor proliferative response in areas of DA denervation raises the possibility that the virus may cause postsynaptic damage. Two families of DA receptors have been distinguished based on similarities in sequence, pharmacology, signaling systems, and the presence or absence of introns: the D1 family, including D1 and D5, and the D2 family, composed of D2, D3, and D4 (Civelli *et al.,* 1991). In D2, D3, and D4 receptor genes, the presence of introns leads to the biosynthesis of several distinct proteins through alternative splicing of pre-mRNA (Schwartz *et al.,* 1993). A unusual feature of BDV molecular biology may lend insight into the basis for the observation that only members of the D2 family of DA receptors appear to be affected. Unlike other nonsegmented, negative-strand RNA animal viruses, BDV uses spliced mRNAs to express its genome (Schneider *et al.,* 1994; Cubitt *et al.,* 1994). BDV may compete with host pre-mRNA species for binding of splicing factors or nucleocytoplasmic RNA transport (Alonso-Caplen *et al.,* 1991). Alternatively, as has been described for another neurotropic virus, HSV-1, BDV may express a viral product that inhibits host cell splicing (Sandri-Goldin *et al.,* 1995). Either mechanism could lead to defective cytoplasmic expression of spliced mRNAs encoding D2 or D3 receptors.

We cannot rule out the possibility that inflammation contributes to the pathogenesis of locomotive hyperactivity in adult infected rats. However, neonatally infected rats do not have central nervous system inflammation yet they also show locomotor hyperactivity. These animals have abnormalities in hippocampal architecture (Bautista *et al.,* 1994; Carbone *et al.,* 1991). Since CA1 and subiculum neurons of the hippocampus project via the fimbria–fornix to N. Acc. (Boeijinga *et al.,* 1993), hippocampal pathology may be associated with N. Acc. dysfunction and hyperactivity (Lipska *et al.,* 1992). It is also possible that hyperactivity in neonatally infected rats reflects intrinsic N. Acc. pathology.

Behavioral pharmacology experiments have demonstrated that conditions of DA excess or enhanced DA receptor sensitivity in the N. Acc. lead to locomotor hyperactivity in rats. Psychostimulant drugs such as *d*-amphetamine and cocaine exert their activation effects by facilitating DA transmission in the N. Acc. (Kelly and Iversen, 1975; Van der Kooy *et al.,* 1983). The supersensitive locomotor response to DA agonists following 6-OHDA lesions of N. Acc. projections has been attributed to the action of DA agonists on increased numbers of DA receptors within the N. Acc. (Staunton *et al.,* 1982). We observe that spontaneous hyperactivity in the BD rat does not readily fit either a pharmacologic or a lesion model of hyperactivity. Although BD rats show partial DA denervation (reduced tissue DA levels and reduced mazindol binding in the N. Acc.), D2 receptors are also decreased. One possible explanation for the discordance between DA-induced behavior and receptor number is that failure of DA reuptake may lead to increased levels of synaptic and extracellular DA and consequent receptor downregulation. Alternatively, D2 receptors may be altered by input from other neurotransmitters. For example, excitatory amino acids (EAA), released from cortical, hippocampal, and amygdala afferents, may augment the postsynaptic effects of DA (Hooks *et al.,* 1994).

EAA projections from the prefrontal cortex share common neuronal targets with DA terminals in the N. Acc. (Sesack and Pickel, 1992). The N. Acc. is a morphologically and biochemically heterogeneous structure in which rostral, core, and shell subregions engage different forebrain and limbic structures. The prelimbic (PL) subfield and the anterior insular (AI) subfield of frontal cortex project preferentially to core and rostral areas (Berendse *et al.,* 1992). Layer 6 pyramidal cells of PL and AI contain high levels of BDV nucleic acid (Solbrig *et al.,* in press). If infection were to cause increased activation or increased glutamate output from cortical-ventral striatal projections, cells could be lost from core and rostral areas as a consequence of glutamate excitotoxicity. A similar relationship between prefrontal cortex projection fibers (Berendse *et al.,* 1992) and compartmental loss of D2 receptors is seen in the dorsal striatum. The ventromedial CP, like the core, is innervated from the prelimbic ventral (PLv) region. It shows a significant reduction in D2 radioligand binding, whereas the dorsolateral CP, which has no prefrontal cortex innervation (Berendse *et al.,* 1992), is spared (Solbrig *et al.,* 1994).

Given the statistically significant changes in DA parameters in core, one might have anticipated more BDV nucleic acid in core than in shell. Although *in situ* hybridization experiments demonstrated higher concentrations of BDV nucleic acid in the shell than in the core, the signal in the shell was both cell- and process-associated. Brainstem monoaminergic nuclei contain high concentrations of BDV nucleic acid (Solbrig *et al.,* 1994). These monoaminergic cells project through the shell to septum and cortex as Zuckerkandl's fascicle. Thus, the increased signal in shell reflects the presence of these passing fibers rather than enhanced infection in cell bodies.

A major effect of BDV infection may be to change the balance between numbers of functional D2 and D3 re-

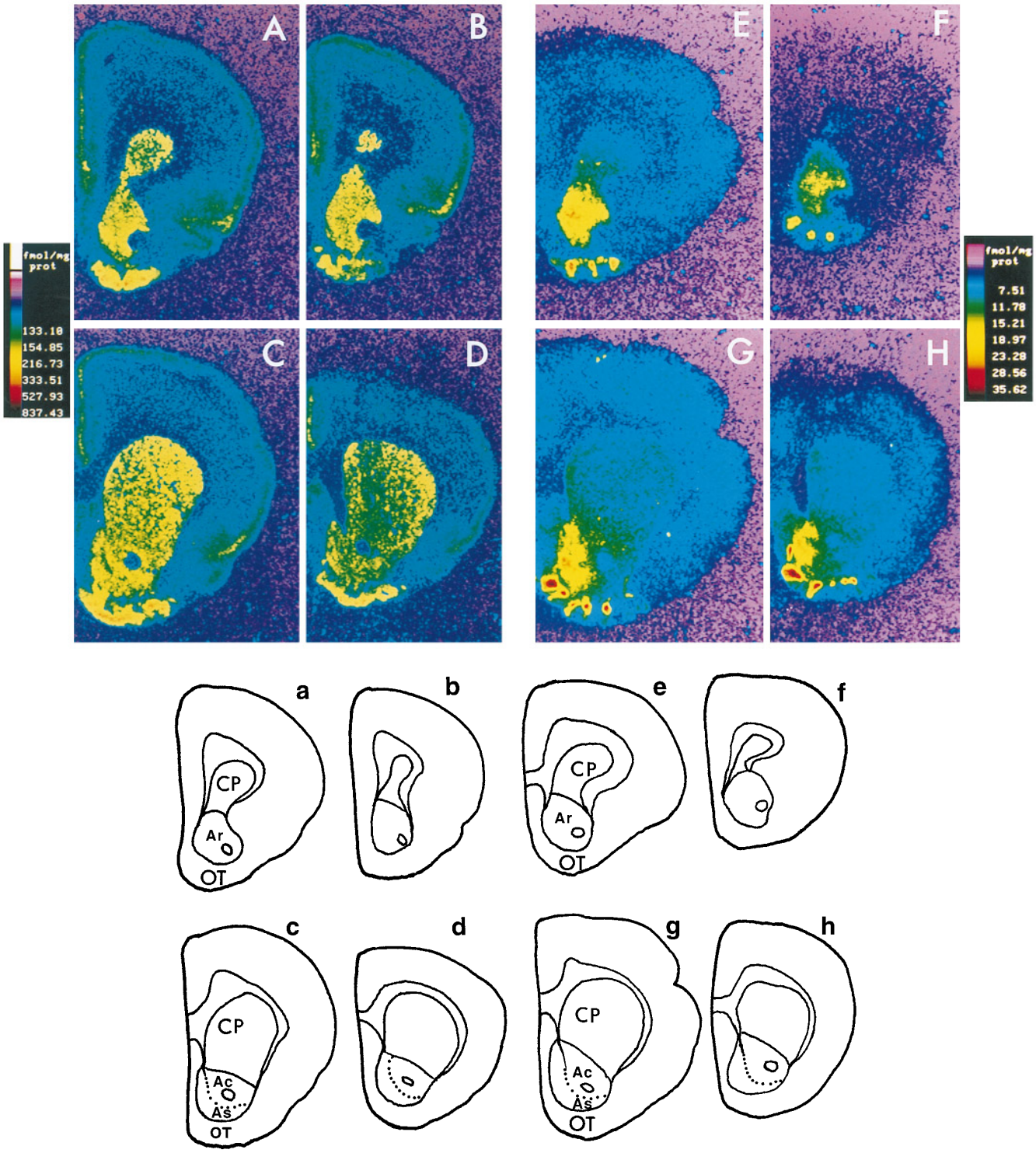

FIG. 3. Dopamine D2 and D3 receptor binding in coronal brain sections from normal rats (A, C, E, G) and BD rats (B, D, F, H). Sections were taken through rostral (A, B, E, F) and core/shell (C, D, G, H) subregions of nucleus accumbens. D2 site labeling by [^{3}H]spiperone (A–D). Specific D2 binding was significantly decreased in the core subregion of BD rats (D). D3 site labeling by ^{125}I-7-OH PIPAT (E–H). Specific D3 binding was significantly decreased in rostral (F) and core (H) subregions of BD rats. Lower case letters in labeled line drawings correspond to upper case letters in autoradiograms. CP, caudate-putamen; Ac, nucleus accumbens core; As, nucleus accumbens shell; Ar, nucleus accumbens rostral; OT, olfactory tubercle.

ceptors in the shell (more limbic) and core (striatal) subregions, resulting in differential modulation of the efficiency of DA transmission in distinct neuronal populations. Experimental evidence indicates that manic-depressive patients have increased numbers of mesolimbic D2 receptors (Pearlson *et al.,* 1995) and schizophrenic patients have increased numbers of both D2 (Seeman *et al.,* 1987) and D3 receptors (Gurevich *et al.,* in press)

independent of neuroleptic administration. The potential for enhanced D2/D3 shell output may be an important pathophysiologic feature that links the BD rat model to human psychiatric diseases.

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