CHARACTERIZATION OF CYTOCHROME P-450 2D1 ACTIVITY IN RAT BRAIN: HIGH-AFFINITY KINETICS FOR DEXTROMETHORPHAN

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ABSTRACT:

We investigated the enzymatic function, stability, and regional distribution of rat brain cytochrome P-450 (CYP) 2D1 activity. CYP2D1 is the homolog of human CYP2D6, a genetically variable enzyme that activates or inactivates many clinical drugs acting on the central nervous system (e.g., antidepressants, monoamine oxidase inhibitors, serotonin uptake inhibitors, and neuroleptics), drugs of abuse (e.g., amphetamine and codeine), neurotoxins (e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1,2,3,4-tetrahydroquinoline), and endogenous neurochemicals (e.g., tryptamine). The CYP2D family has been identified in rodent, canine, and primate brain. Conversion of dextromethorphan to dextrorphan by rat brain membranes was assayed by HPLC and was dependent on NADPH, protein concentration, and incubation time. Significant loss of activity was observed in some homogenizing buffers and after freezing of whole tissues or membrane preparations. Dextromethorphan (0.5–640 μM) metabolism was mediated by high- and low-affinity enzyme systems; \( K_m \) was 2.7 ± 2.6 and \( K_m \) was 757 ± 156 μM (\( n = 3 \) rats, mean ± S.E.). The enzyme activity was significantly (\( p < .01 \)) and stereoselectively inhibited by CYP2D1 inhibitors quinine and quinidine (not by CYP2C or CYP3A inhibitors), and by anti-CYP2D6 peptide antisera (not by anti-CYP2C, -CYP2B, or -CYP3A antibodies). The enzymatic activity demonstrated significant brain regional variation (\( n = 10 \) regions, \( p < .001 \)). These data characterize CYP2D1-mediated dextromethorphan metabolism in rat brain and suggest that localized metabolism of other CYP2D1 substrates (drugs, neurotoxins, and possibly endogenous compounds) within the brain will occur. In humans, CYP2D6 is genetically polymorphic; the variable expression of brain CYP2D6 may result in interindividual differences in central drug and neurotoxin metabolism, possibly contributing to interindividual differences in drug effects and neurotoxicity.

Cytochrome P-450 (CYP)\(^1\) 2D6 is genetically polymorphic, absent in 5 to 10% of the Caucasian population (Gonzalez and Meyer, 1991). This polymorphism causes impaired metabolism of more than 20 centrally acting drugs including tricyclic antidepressants, monoamine oxidase inhibitors, serotonin uptake inhibitors and neuroleptics; as well as many drugs of abuse, such as codeine, hydrocodeine, dextromethorphan (DEX), and amphetamines (reviewed in Parkinson, 1996). CYP2D6 and the rat homolog CYP2D1 have also been shown to metabolize carcinogens and neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Fonne-Pfister et al., 1987; Jiménez-Jiménez et al., 1991) and 1,2,3,4-tetrahydroquinoline (Ohta et al., 1990). The CYP2D family may also be involved in the metabolism of the endogenous neurochemical substrate tryptamine (Martínez et al., 1996). Cytochrome P-450 (CYP)\(^1\) 2D6 is genetically polymorphic, absent in 5 to 10% of the Caucasian population (Gonzalez and Meyer, 1991). This polymorphism causes impaired metabolism of more than 20 centrally acting drugs including tricyclic antidepressants, monoamine oxidase inhibitors, serotonin uptake inhibitors and neuroleptics; as well as many drugs of abuse, such as codeine, hydrocodeine, dextromethorphan (DEX), and amphetamines (reviewed in Parkinson, 1996). CYP2D6 and the rat homolog CYP2D1 have also been shown to metabolize carcinogens and neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Fonne-Pfister et al., 1987; Jiménez-Jiménez et al., 1991) and 1,2,3,4-tetrahydroquinoline (Ohta et al., 1990). The CYP2D family may also be involved in the metabolism of the endogenous neurochemical substrate tryptamine (Martínez et al., 1996). Supported in part by National Institute on Drug Abuse Grant DA06889, Medical Research Council of Canada Grant MT14173, and the Center for Addictions and Mental Health, Canada. Parts of this work were previously presented at the following meetings: X'th International Symposium on Microsomes and Drug Oxidations, 1996; Congress on the Problems of Drug Dependence, 1996; and Society for Neuroscience meeting 1995. \(^1\)Abbreviations used are: CYP, cytochrome P-450; DEX, dextromethorphan; DOR, dextrorphan; CNS, central nervous system; QN, quinine; QD, quinidine; SPZ, sulfaphenazole; TRL, troleandomycin; ACSF, artificial cerebrospinal fluid.

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distribution of CYP2D1-mediated catalytic activity in 10 brain regions.

Materials and Methods

Chemicals and Antibodies. DEX·HBr, quinine (QN), quinidine (QD), sulfaphenazole (SPZ), tromethamine (TRL), and butorphanol (internal standard) were purchased from Sigma Chemical Co. (St. Louis, MO). DOR tartrate was kindly provided by Hoffmann-La Roche (Nutley, NJ). Antibodies against CYP2B1, CYP2B6, CYP2C11, CYP2C9/19, CYP2D6, CYP3A2, and CYP3A4 were obtained from GENTEST (Woburn, MA). An additional antibody against CYP2D6 was also kindly provided by A. Cribb and Merck-Frost (West Point, PA; Cribb et al., 1995).

Animals. Male Wistar rats (250–300 g) from Charles River (Québec, Canada) were used. The research protocol was approved by the Animal Care Committee of the Addiction Research Foundation of Ontario, Canada. Rats were sacrificed by decapitation, and the brains and livers were immediately removed and washed with ice-cold 0.9% (w/v) sodium chloride. The brain regions were identified and dissected on ice [according to Paxinos and Watson’s stereotaxic atlas of the rat brain (1986)] and homogenized in an ice-cold artificial cerebrospinal fluid solution (ACSF; 126 mM NaCl, 2.68 mM KCl, 1 mM NaHPO₄, 0.88 mM MgSO₄, 22 mM NaHCO₃, 1.15% (w/v) KCl, 20% (v/v) glycerol] designed for homogenizing and assay- ing CYPs in rat brain membranes (Ravindranath and Anandatheerthavarada, 1990) was also tested to determine which buffer system provided the best retention of CYP2D1 activity. We also investigated whether there was loss of enzymatic activity after storage of intact cerebellar tissue and/or cerebellar membranes (in both homogenization buffers).

CYPs in brain differ from those in liver in their relative subcellular localization, with more cerebral CYP in the mitochondrial versus microsomal fractions (Ghersi-Egea et al., 1993; Bhagwat et al., 1995). In preliminary studies we detected CYP2D1 activity in cerebellar mitochondrial, microsomal, plasma, and nuclear membranes (prepared according to Ghersi-Egea et al., 1993). The amount of CYP and DEX metabolism varied considerably between membrane types and among different brain regions. Total membrane fractions were prepared by centrifuging the crude tissue homogenate at 100,000 g for 60 min and pellets were removed and washed with ice-cold 0.9% (w/v) sodium chloride. The mitochondria and/or preimmune serum (80 µl total) were preincubated with cerebellar membranes at room temperature for 30 min, then with DEX at 37°C for 120 min. In test samples specific anti-CYP sera were used to replace preimmune serum (maintaining a total of 80 µl/sample for control and test samples). QN, QD, SPZ, and TRL are widely used and selective chemical inhibitors of CYP2D1 (QN and QD; Broly et al., 1989; Kobayashi et al., 1989), the CYP2C family (SPZ; Veronese et al., 1990), and the CYP3A family (TRL; Werner et al., 1995), respectively.

DOR, the O-demethylated metabolite of DEX, was extracted and measured by the method of Otton et al. (1993) with minor modifications as follows. Briefly, samples were extracted with 3 ml of hexane/ether (4:1, v/v), vortexed for 10 min, and centrifuged at 3000 rpm for 5 min; then 2.4 ml of the top (organic) layer was mixed with 200 µl of 0.01 N HCl. After vortexing for 5 min and centrifugation (3000 rpm for 5 min) the organic phase was removed. After the second extraction, 50 µl of the aqueous phase was injected onto an HPLC column.

The HPLC system (model 1050, Hewlett-Packard) consisted of a phenyl column (150 × 4.6 mm) with an electrochemical detector. Samples were eluted at a flow rate of 1 ml/min with a mobile phase of acetonitrile/10 mM KH₂PO₄ (24/76, v/v, pH 3.8) containing 1 mM heptanesulfonic acid and monitored by the detector setting at +0.3 V for the first electrode and +0.76 V for the second electrode. DOR eluted at 8.11 min and the internal standard, butorphanol, at 10.90 min. Samples were quantified with a calibration curve of DOR/butorphanol versus concentration of DOR. The assay was linear from 0.5 to 2000 ng (2–7770 pmol) DOR/ml.

Data Analysis. K_m and V_max values were calculated by linear regression and plotted with the Eadie-Hofstee equation. Significance was determined by ANOVA followed by Student’s t test, to evaluate the differences in DOR formation between the control and treatment groups and among different brain regions.

Results

A rapid, sensitive, and selective HPLC assay was developed; the sensitivity limit was 4 pmol DOR/ml of the assay mixture. DOR was the only metabolite detected after DEX incubations with rat brain membranes. At a concentration of 40 pmol/ml, the overall recovery of DOR was 72.3 ± 7.8%. The inter- and intraday coefficients of variation for DOR were 5.6 and 7.3%, respectively. This assay adaptation allows us to measure DOR formation from brain membranes in vitro at low micromolar DEX substrate concentrations (0.5 µM).

Storage of dissected intact cerebellum at −70°C for 7 or 14 days followed by tissue homogenization, membrane preparation, and incubations resulted in a 59 or 58% loss of enzyme activity relative to the activity observed without freezing (Fig. 1A). DEX metabolic activity was found in microsomal, mitochondrial, and the 1000g pellet membranes (Fig. 1B). However, due to rat brain enzymatic stability issues (see below) all subsequent assays were performed on the same day as the animals were sacrificed. Therefore for this initial characterization

![Fig. 1. Influence of tissue storage and membrane preparation on DEX metabolism.](image-url)
of CYP2D1 activity, the experiments were performed using total membrane preparations rather than differing membrane fractions.

We tested ACSF and a Tris buffer (previously designed to preserve brain CYP activity [Ravindranath and Anandatheerthavarada, 1990]) as homogenization buffers for the rat brain membranes. Significantly \((p < .05)\) higher CYP2D1 activity (DEX at 40 \(\mu M\)) was observed after homogenization and incubation in ACSF (7.9 ± 2.2 pmol DOR/mg protein/h) compared with the Tris buffer system (1.2 ± 0.6 pmol DOR/mg protein/h, Fig. 2A). In addition, when the Tris buffer was used to homogenize the tissue, but the assay incubations were carried out in the ACSF buffer, there was also a considerable decline in activity (54\%, Fig. 2B). There was no significant decrease in cerebellar CYP2D1 activity when the tissue was flash frozen before homogenization in either buffer system (Fig. 2B). However, there was a significant loss of CYP2D1 activity after storage of the cerebellar membranes for 7 days at −70°C in either homogenization buffer system (Fig. 2B; 39\% in ACSF, \(p < .01\); 48\% in Tris, \(p = .05\)). The loss of activity from membrane storage at −70°C for 5 or 14 days was consistent across five brain regions tested and appeared to be already maximal at five days of freezing (Fig. 2C). As a result of these studies (Figs. 1 and 2) all subsequent experiments were performed with total membrane preparations rather than differing membrane fractions.

No DOR was formed (below detection limit) when heat-denatured \((100°C, 20\) min) total membranes were used as the enzyme source (Fig. 3A). Incubation of DEX (40, 20, and 10 \(\mu M\)) with brain cerebellar membranes in the absence of NADPH significantly \((p > .01)\) decreased the DOR formation (82–96\%; Fig. 3A). Initial determination of DOR formation over a range of protein concentrations and incubation times is shown in Fig. 3B. This was further tested at DEX concentrations of 10 and 40 \(\mu M\) with 0 to 5 mg protein/ml at 120 min and 0 to 180 min at 3 mg protein/ml, confirming that DOR formation was linear as a function of membrane protein concentration, from 0 to 3 mg/ml, up to 120 min incubation time. Slight nonlinearity at 3 mg/ml at 180 min in some brain regions could result in kinetic plots that modestly overestimate \(K_m\) values.

Kinetics of DEX conversion to DOR by rat brain cerebellar membranes was determined by varying DEX concentrations from 0.5 to 640 \(\mu M\) \((n = 3\) separate animals assayed in duplicate). Eadie-Hofstee plots demonstrate the two enzymes involved in DOR formation with \(K_m^1 2.7 ± 0.6 \mu M\) (mean ± S.E.) and \(V_{max} 2.8 ± 0.5 \text{ pmol/mg protein/h}\) and \(K_m^2 757 ± 157 \mu M\) and \(V_{max2} 136 ± 12 \text{ pmol/mg protein/h}\). Figure 4 shows typical data from one animal. In addition, from the individual data one can also assess the relative contribution of the high-affinity \((\text{low } K_m)\) component to the metabolism at different concentrations of substrate. These data predict that at 10, 20, and 40 \(\mu M\) DEX the high-affinity \((\text{low } K_m)\) enzyme contributes 53 ± 8, 39 ± 7, and 27 ± 6\%, respectively.

DOR formation by rat cerebellar membranes is stereoselectively inhibited by the CYP2D1 inhibitors QN and QD (Kobayashi et al., 1989; Fig. 5A). The activity in cerebellar membranes was inhibited more by QN than by QD at each substrate concentration (Fig. 5A), consistent with rat liver CYP2D1 inhibition (Kobayashi et al., 1989). In addition, neither hepatic nor brain activity was inhibited by SPZ, a CYP2C family inhibitor (Veronese et al., 1990) or TRL, a CYP3A family inhibitor (Werner et al., 1995; Fig. 5, B and D). Furthermore, anti-human CYP2D6 peptide antiserum (20 \(\mu l\)) selectively inhibited DOR formation by 54\%; the reaction was not inhibited by antibodies against rat CYP2C11, CYP2B1, or CYP3A2 (shown in Fig. 5C) or by antibodies against human CYP2C9/18/19 or CYP2B6 (data not shown).

Figure 6 illustrates that the CYP2D1 activity (DEX 10 \(\mu M\)) was distributed unevenly in 10 different regions of rat brain. The data in Fig. 6 show each region as a fraction of the cerebellum; the significance of differences between regions (\(p\) values in Student \(t\) tests) for the transformed data and the raw data can be found in Table 1. The highest catalytic activity was found in the cerebellum, while the lowest was found in the striatum (32\% of cerebellum), demonstrating a 3-fold variation in CYP2D1 activity.

Discussion

Our studies clearly indicated that the ACSF was superior as a homogenization and assay buffer for CYP2D1 relative to the Tris buffer system, which was designed to preserve brain CYPs (Ravindranath and Anandatheerthavarada, 1990). In addition, our storage studies indicated that there was significant loss of enzymatic activity when tissue or membranes were stored at −70°C. In concurrent studies, immunoreactive CYP2D2, as assessed by Western blotting, did not decrease significantly with freezing (S.M. and R.F.T., unpublished data) suggesting that the loss of enzymatic activity is unrelated to loss of immunoreactive protein. If these tissue and membrane freezing and storage issues are pertinent to all brain CYPs, it may account for the relative prevalence of literature reporting detection of CYPs in brain by using immunoreactivity techniques, while the respective enzymatic activities have been more difficult to detect or quantify. Our studies indicated that a small portion of the activity

![Fig. 2. Influence of homogenization buffers and tissue storage on DEX metabolism in rat brain membranes.](image-url)
remained in the absence of externally added NADPH (Fig. 3A). In concurrent studies examining the CYP2D1-mediated metabolism of hydrocodone and amphetamine, incubation in the absence of NADPH has resulted in undetectable product formation. This suggests that the DEX metabolism measured may be utilizing endogenous NADPH or NADH as the electron donor. Previous studies of enzyme activities in brain mitochondrial fractions suggest that the mitochondrial CYPs can use the endogenous NADPH-generating system or an alternative electron donor (Bhagwat et al., 1995). Alternatively the DEX metabolism in the absence of NADPH may be due to a low-affinity enzyme less strictly dependent on NADPH.

Human and rat liver CYP2D1/6 have similar $K_{m1}$ values for DEX of 5 $\mu$M (Dayer et al., 1989; Martı́nez et al., 1997) and 2.5 $\mu$M (Kerry et al., 1993), respectively. Rat liver also has a lower-affinity enzyme with a $K_{m2}$ of 158 $\mu$M; 98% of total intrinsic clearance was contributed by the high-affinity $K_{m1}$ site (Kerry et al., 1993). In the present experiments we found that the brain high-affinity enzyme site had a $K_{m}$ value very similar to that found in liver (rat brain $K_{m1}$ 2.7 ± 0.6 $\mu$M; rat liver $K_{m1}$ 2.5 $\mu$M), whereas the $K_{m}$ value of the low-affinity site was considerably higher than that found in liver (rat brain $K_{m2}$ 757 ± 157 $\mu$M; rat liver $K_{m2}$ 158 $\mu$M), suggesting that the low-affinity enzymes in brain and liver were different CYP isozymes or perhaps that the $K_{m}$ value was altered by different relative reductase levels. Jolivalt et al. (1995) published $K_{m}$ values of 0.1 and 0.4 mM for DEX metabolism in male Sprague-Dawley rat liver and brain, respectively, as well as very high values for QD inhibition of the DEX metabolism in liver ($K_{i}$ 1.2 mM) and brain ($K_{i}$ 1.7 mM). These data suggest that Jolivalt et al. (1995) were in fact studying a low-affinity enzyme, perhaps CYP3A, and not the high-affinity ($K_{m1}$) CYP2D1.

The stereoisomers QN and QD are widely used as selective inhibitors of rat CYP2D1 and human CYP2D6 and are frequently employed to identify the involvement of this isozyme in specific metabolic pathways (Broly et al., 1989; Gonzalez and Meyer, 1991). Consistent with the greater potency of QN versus QD to inhibit rat liver CYP2D1 (Kobayashi et al., 1989), QN was more potent in selectively inhibiting DOR formation by rat brain membranes (Fig. 5A), providing further evidence that the activity measured was catalyzed by CYP2D1. No inhibition of liver or brain DEX metabolism was observed with SPZ or TRL (Fig. 5, B and D). Furthermore, selective inhibition of DOR formation by antibodies to CYP2D6, but not by antibodies to CYP2C11, CYP2B, or CYP3A2, was demonstrated (Fig. 5C). The partial inhibition of the brain-mediated DEX metabolism by the anti-CYP2D1 chemical and antibody inhibitors is consistent with the portion of the metabolism mediated by the high-affinity site (52 and 39% at 10 and 20 $\mu$M, respectively). These data suggest that the high-affinity site that was determined in these studies is mediated by CYP2D1, not by members of the CYP2C or CYP3A families.

In canine brain, we have demonstrated large variation in the CYP2D1/6-mediated sparteine metabolism (Tyndale et al., 1991). Immunological studies have also identified regionally variable expression of brain immunoreactive CYP2D (Norris et al., 1996). Our results with DEX metabolism (10 $\mu$M) in rat brain also demonstrate significant regional variation in CYP2D1 activity (Table 1). The rank order of the activity among brain regions differs from that found in the dog (Tyndale et al., 1991), suggesting that there may be species differences in the regional expression of this enzyme in brain.

CNS metabolism of drugs is unlikely to affect the overall biotransformation of most drugs quantitatively; however, it may have pro-
found effects on localized drug and metabolite concentrations (Britto and Wedlund, 1992). This could have a number of potential consequences. For example, metabolism of codeine, which is O-demethylated by CYP2D1/6 to morphine within the brain, alters the onset of analgesia (Chen et al., 1990). There is also some evidence for the endogenous production of morphine from codeine and ovipavine from thebaine, within the brain; these pathways are mediated at least in part by CYP2D1 (Kodaira and Spector, 1988). This suggests that brain CYP2D1/6 may play an endogenous role in morphine production and control of pain.

Many studies have demonstrated the oxidation of amphetamines by CYP2D1/6 and the metabolism of amphetamines by brain tissue (Lin et al., 1992; Tucker et al., 1994; Lin et al., 1995; Chu et al., 1996). Formation of false neurotransmitters, reactive metabolites, and metabolites with distinct pharmacology, as well as tolerance and hyperthermic and neurotoxic effects, have all been attributed to the relative and localized CNS concentrations of the amphetamine and its hydroxy-metabolites (Tucker et al., 1994; Colado and Williams, 1995; Chu et al., 1996). These reports suggest that the polymorphic expression of CYP2D6 in human brain may be one component of risk/protection in the dependence on and toxicity of amphetamines such as “ecstasy.”

Many articles have suggested the involvement of deficient CYP2D6 in the etiology of Parkinson’s disease; some studies support the association, whereas others do not (Armstrong et al., 1992; Smith et al., 1992). In addition, biochemical studies indicate that known neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1,2,3,4-tetrahydroquinoline are metabolized by CYP2D1/6 by what is thought to be a detoxifying pathway (Fonne-Pfister et al., 1987; Ohta et al., 1990; Jiménez-Jiménez et al., 1991; Gilham et al., 1997). Because Parkinson’s disease is characterized by degeneration of neurons, these results suggest the possibility that a functional brain CYP2D1/6 may be protective against Parkinson’s disease. This led to our original studies identifying CYP2D1/6 in dog and human brain and demonstrating a substantial substrate overlap with the dopamine transporter (Niznik et al., 1990; Tyndale et al., 1991). Our present findings, identifying and characterizing CYP2D1 in rat brain, further suggest that one role for brain CYP2D1/6 may be in the removal and metabolism of drugs, neurotoxins, and endogenous catecholamines from the synaptic cleft, in cooperation with the dopamine transporter.
Recent findings have identified the CYP2D6-mediated metabolism of tyramine, octopamine, and norepinephrine, and epinephrine, respectively (Hiroi et al., 1998). Tryptamine has also been described as a substrate for CYP2D1 (Martínez et al., 1997).

These findings suggest that central metabolism of endogenous trace amines by polymorphic CYP2D6 may be related to the polymorphic differences in personality that have been observed (Bertilsson et al., 1989; Llerena et al., 1993). There may also be a role for brain CYP2D6 in neural development that has subsequent effects on the development of personality. Our data indicate that CYP2D1 is expressed in a number of brain regions; lack of metabolism in some or all of these regions in human CYP2D6 poor metabolizers may contribute to these observed differences in personality. Polymorphic creation of neurotransmitters by brain CYP2D6 may also affect other aspects of normal central function and neurotoxicological risk in ways that remain to be elucidated.

In conclusion, we have identified a rat brain enzyme that metabolizes the prototype CYP2D1 probe drug DEX with saturable kinetics and has a high-affinity $K_m$ similar to that of rat liver CYP2D1. It is also inhibited selectively by QN and the anti-CYP2D6 peptide antiserum. These characteristics are very similar to those identified for CYP2D1/6 in rat and human liver (Kobayashi et al., 1989; Tyndale et al., 1992).

In the brain, CYP2D1/6 is expressed in many different brain regions, with the highest activity in cerebellum. We postulate that polymorphic and regional brain expression of CYP2D1/6 may play a key role in interindividual differences in the effects of CNS acting drugs, neurotoxins, and/or endogenous compounds. The characterization of rat brain CYP2D1 suggests that the rat may be a good animal model for testing the role of brain CYP2D1 activity in vivo and for determining the influence of brain CYP2D1 metabolism on drug action and toxicity.

Acknowledgments. We thank Dr. A.E. Cribb for anti-CYP2D6 peptide antiserum, and Dr. Peter Wu, Mae Kwan, and Helma Nolte for their assistance.

References


Differences in CYP2D1 activity (DEX 10 µM) among 10 brain regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Velocity $^a$</th>
<th>COB</th>
<th>HC</th>
<th>BS</th>
<th>SC</th>
<th>PO</th>
<th>TC</th>
<th>FC</th>
<th>PC</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum (CB)</td>
<td>2.96 ± 0.58</td>
<td>0.093</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
<td>&lt;0.001$^b$</td>
</tr>
<tr>
<td>Occipital bulb (OB)</td>
<td>1.96 ± 0.44</td>
<td>0.016$^b$</td>
<td>0.019$^b$</td>
<td>0.027$^b$</td>
<td>0.028$^b$</td>
<td>0.032$^b$</td>
<td>0.038$^b$</td>
<td>0.036$^b$</td>
<td>0.036$^b$</td>
<td>0.037$^b$</td>
</tr>
<tr>
<td>Hippocampus (HC)</td>
<td>1.71 ± 0.52</td>
<td>&lt;0.001$^b$</td>
<td>0.145</td>
<td>0.293</td>
<td>0.078</td>
<td>0.039$^b$</td>
<td>0.037$^b$</td>
<td>0.040$^b$</td>
<td>0.019$^b$</td>
<td>0.019$^b$</td>
</tr>
<tr>
<td>Brain stem (BS)</td>
<td>1.55 ± 0.28</td>
<td>0.018$^b$</td>
<td>0.106</td>
<td>0.573</td>
<td>0.245</td>
<td>0.005$^b$</td>
<td>0.002$^b$</td>
<td>0.001$^b$</td>
<td>0.002$^b$</td>
<td>0.003$^b$</td>
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<tr>
<td>Spinal cord (SC)</td>
<td>1.40 ± 0.24</td>
<td>0.052</td>
<td>0.641</td>
<td>0.724</td>
<td>0.457</td>
<td>0.460</td>
<td>0.436</td>
<td>0.436</td>
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<tr>
<td>Pons (PO)</td>
<td>1.23 ± 0.32</td>
<td>0.008$^b$</td>
<td>0.020$^b$</td>
<td>0.108</td>
<td>0.006$^b$</td>
<td>0.018</td>
<td>0.435</td>
<td>0.426</td>
<td>0.412</td>
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<tr>
<td>Temporal cortex (TC)</td>
<td>1.21 ± 0.30</td>
<td>0.011$^b$</td>
<td>0.027$^b$</td>
<td>0.114</td>
<td>0.007$^b$</td>
<td>0.159</td>
<td>0.972</td>
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<td>Frontal cortex (FC)</td>
<td>1.10 ± 0.24</td>
<td>0.014$^b$</td>
<td>0.040$^b$</td>
<td>0.130</td>
<td>0.019$^b$</td>
<td>0.429</td>
<td>0.258</td>
<td>0.823</td>
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<tr>
<td>Parietal cortex (PC)</td>
<td>1.09 ± 0.25</td>
<td>0.013$^b$</td>
<td>0.040$^b$</td>
<td>0.148</td>
<td>0.243</td>
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<td>0.606</td>
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<td>Striatum (ST)</td>
<td>1.09 ± 0.29</td>
<td>0.010$^b$</td>
<td>0.019$^b$</td>
<td>0.087</td>
<td>0.003$^b$</td>
<td>0.024$^b$</td>
<td>0.054</td>
<td>0.024$^b$</td>
<td>0.087</td>
<td>0.294</td>
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</table>

$^a$ Mean activity ± S.E. pmol dextrophan/mg protein/h (n = 4 rats assayed separately).

$^b$ Significance at $p < 0.05$.
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