Chronic nicotine treatment induces rat CYP2D in the brain but not in the liver: an investigation of induction and time course

*2006 Innovations in Neuropsychopharmacology Award**

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Objectives: CYP2D6 levels are higher in many brain regions of human smokers in comparison with nonsmokers. We have shown that CYP2D is expressed in rat brain regions and that enzyme activities correlate with protein and messenger ribonucleic acid (mRNA) levels. The aims of this study were to investigate whether nicotine can induce rat brain CYP2D, to determine the recovery time course of the induction and to investigate the mechanism of induction through measuring mRNA levels over time. **Methods:** Rats were either treated once with either saline or nicotine (1 mg base/kg, subcutaneous and sacrificed 8 hours after the treatment or treated daily for 7 days and sacrificed 0.5–24 hours after the last injection. The CYP2D protein and mRNA levels were assessed by immunoblotting, immunocytochemistry and slot blotting. **Results:** There were no changes in brain CYP2D levels after a single nicotine injection. Following chronic nicotine treatment, levels were maximal at 8 hours and returned to control levels by 12 hours after nicotine treatment in all 3 regions assessed. At 8 hours after nicotine treatment, CYP2D levels were significantly ($p < 0.05$) higher than levels in saline-treated control animals in the cerebellum (1.4-fold), hippocampus (1.3-fold) and striatum (3.2-fold); they tended to be higher in the frontal cortex, brainstem and thalamus. Induction was specific to brain region and cell, for example, in some striatal neurons and in neurons in the cerebellar granular layer and white matter. At no time was there any increase in brain CYP2D mRNA levels. Hepatic CYP2D levels were unchanged at all times tested. **Conclusion:** Chronic nicotine treatment induced CYP2D enzymes in rat brain but not rat liver. The induction was maximal 8 hours after the last injection and did not involve alterations in mRNA, indicating a posttranscriptional mechanism. These findings suggest that, in humans exposed to nicotine, response to centrally acting drugs metabolized by CYP2D, susceptibility to neurotoxins either activated or inactivated by CYP2D and the general homeostasis of endogenous neurochemicals metabolized by CYP2D may be affected, owing to increased CYP2D in the brain.

Objectifs : Les concentrations de CYP2D6 sont plus élevées dans beaucoup de régions du cerveau des fumeurs humains que chez les non-fumeurs. Nous avons démontré que la CYP2D est exprimée dans des régions du cerveau du rat et qu'il y a corrélation entre les activités enzymatiques et les concentrations de protéines et d'acide ribonucléique messager (ARNm). Cette étude visait à déterminer si la nicotine peut induire la CYP2D dans le cerveau de rat, à déterminer l'évolution du temps de rétablissement de l'induction et à étudier le mécanisme d'induction en mesurant les concentrations d'ARNm au fil du temps. **Méthodes :** Les rats ont reçu un seul traitement constitué d'une solution physiologique ou de nicotine (1 mg référence/kg) par voie sous-cutanée et ont été sacrifiés 8 heures après le traite-

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Medical subject headings: animals; cytochrome P450 enzyme system; nicotine; brain; central nervous system; metabolism; smoking; Parkinson disease.

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ment ou reçu un traitement quotidien pendant 7 jours et ont été sacrifiés de 0,5 à 24 heures après la dernière injection. On a évalué les concentrations de protéine CYP2D et d'ARNm par les techniques immunoblot et «slot blot», ainsi que par immunocytochimie. **Résultats :** Les concentrations de CYP2D dans le cerveau n'ont pas changé après une seule injection de nicotine. Après le traitement chronique à la nicotine, les concentrations ont atteint leur niveau maximal à 8 heures et sont revenues aux niveaux témoins 12 heures après le traitement à la nicotine dans les trois régions évaluées. Huit heures après le traitement à la nicotine, les concentrations de CYP2D étaient beaucoup plus élevées ($p < 0,05$) que chez les animaux témoins qui ont reçu une solution physiologique, dans le cervelet (facteur de 1,4), l'hippocampe (facteur de 1,3) et le corps strié (facteur de 3,2); elles avaient tendance à être plus élevées dans le cortex frontal, le tronc cérébral et le thalamus. L'induction était spécifique à la région du cerveau et à la cellule : p. ex., dans certains neurones du corps strié et dans des neurones de la couche granulaire du cervelet et de la matière blanche. Les concentrations d'ARNm et de CYP2D dans le cerveau n'ont jamais augmenté. La concentration de CYP2D dans le foie n'a pas changé pendant tous les tests. **Conclusion :** Le traitement chronique à la nicotine a induit les enzymes de la CYP2D dans le cerveau du rat, mais non dans le foie. L'induction a atteint son point maximal 8 heures après la dernière injection et n'a pas entraîné d'altération de l'ARNm, ce qui indique l'existence d'un mécanisme posttranscriptionnel. Ces constatations indiquent que, chez les êtres humains exposés à la nicotine, l'élévation de la concentration de CYP2D dans le cerveau peut avoir un effet sur la réponse à des drogues à action centrale métabolisée par la CYP2D, la susceptibilité aux neurotoxines activées ou inactivées par la CYP2D et l'homéostase générale de produits neurochimiques androgènes métabolisés par la CYP2D.

Introduction

Cytochrome P450s belong to a superfamily of heme-thiolate enzymes that are principally responsible for biotransformation of a wide diversity of xenobiotics (e.g., clinical drugs and toxins) and endogenous compounds (e.g., catecholamines, neurosteroids and bile acids).[1,2] Human CYP2D6 is estimated to be involved in the metabolism of 20%~30% of therapeutic drugs, making it a clinically significant enzyme.[3] The *CYP2D6* gene is highly polymorphic, and more than 100 different alleles have been identified (www.imm.ki.se /CYPalleles/cyp2d6.htm). Many of the substrates of CYP2D6 are drugs that act on the central nervous system,[4] such as the analgesic codeine; the tricyclic antidepressants amitriptyline and imipramine; the antidepressants fluoxitine, paroxetine and citalopram; the antipsychotics haloperidol and risperidone; and the recreational drugs methoxyamphetamine and methylenedioxymethamphetamine. CYP2D6 may contribute to several endogenous pathways, including the conversion of 4-methoxyphenylethylamine to tyramine[5] and then to dopamine,[5,6] the conversion of 5-methoxytryptamine to serotonin[7,8] and the metabolism of β-carbolines[9] and neurosteroids.[10,11] CYP2D6 can also inactivate neurotoxins, for example, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)[12] and the tetrahydroisoquinolines,[13] both associated with development of symptoms of Parkinson disease.[14]

Rat brain membranes can O-demethylate codeine[15] and dextromethorphan[16,17] and can hydroxylate amphetamines.[18] We have shown that there is a correlation between levels of rat brain CYP2D protein, ribonucleic acid (RNA) and enzymatic activity.[17] CYP2D6 protein,[19–21] messenger RNA (mRNA)[19,22,23] and enzymatic activity[24] have been identified in the human brain. CYP2D6 is expressed heterogeneously among brain regions and cells, creating microenvironments where CYP2D6 expression may be as high as hepatic levels, which may allow for in situ CYP2D6-mediated metabolism of centrally acting drugs, neurotoxins and endogenous neurochemicals.[25] Induction of brain CYP2D6 would increase local brain metabolism, which might contribute to the interindividual variation in response to centrally acting drugs that cannot be explained by drug plasma levels, as well as altering drug–drug interactions, susceptibility to neurotoxins and endogenous pathways.

Human smokers have higher levels of brain CYP2D6[19,26] but unchanged levels of hepatic CYP2D6.[27] Of the compounds in tobacco smoke, we and others have found that chronic nicotine can induce rodent brain CYP2B1[28,29] and CYP2E1.[30–32] If brain CYP2D is induced by nicotine, this could affect brain CYP2D6 metabolism in a large portion of the population — not only smokers but also those exposed to environmental tobacco smoke and those undergoing nicotine replacement therapy. The aims of this study were to investigate whether nicotine can induce rat brain CYP2D, to determine the recovery time for the induction and to investigate the mechanism of induction by measuring mRNA levels over time. If nicotine does increase brain CYP2D, this provides an animal model where the effect of altering brain CYP2D levels on the metabolism of centrally acting substrates can be investigated.

Methods

Adult male Wistar rats (250–300 g; Charles River, St. Constant, Que.) were housed 2 per cage with free access to food and water and maintained on a 12-hour artificial light–dark cycle (light on at 6 am) throughout the study period. All experimental procedures were carried out in accordance with the Canadian guidelines for the care and use of laboratory animals and were approved by the University of Toronto's Animal Care Committee.

Drug treatment

Nicotine-treated rats were injected subcutaneously with nicotine bitartrate (in sterile saline, pH 7.4) at 1 mg base/kg[29]; control rats were injected with saline. For the chronic post-treatment time course, rats were treated with saline or nicotine once daily for 7 days and sacrificed at 0.5, 2, 4, 8, 12, 18 and 24 hours after the last injection. For the acute study, rats

were injected with nicotine or saline once and sacrificed 8 hours after treatment. Animals were sacrificed by decapitation, and the brains were rapidly removed and dissected into regions. The samples for immunoblotting were frozen immediately in liquid nitrogen and stored at –80°C. The tissues used for RNA slot blot were stored in RNA*later* (Ambion Inc., Austin, Tex.) according to the manufacturer's protocol. There were no significant differences in body weight between rats administered nicotine or rats administered saline for 7 days.

Immunoblotting

Total brain membranes were prepared because brain CYPs are present in multiple membrane fractions.[33] The brain regions were manually homogenized with the use of glass homogenizers in 100 mM Tris (hydroxymethyl) aminomethane hydrochloride (Tris HCl) (pH 7.6 at 4°C) with 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.32 M sucrose and 0.1 mM dithiothreitol on ice. Homogenates were centrifuged twice at 3000 *g* for 5 minutes to remove cellular and nuclear debris, and then the supernatants were centrifuged at 110 000 *g* at 4°C for 90 minutes. Liver microsomes were prepared as described previously.[34] The resulting membrane pellets were resuspended in the storage solution, consisting of 100 mM Tris HCl (pH 7.4), 0.1 mM EDTA, 0.1 mM dithiothreitol, 1.15% w/v potassium chloride and 20% v/v glycerol, and stored in aliquots at –80°C until used.

The protein content was assayed according to Bradford's method, with the use of a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Mississauga, Ont.). Membrane proteins were separated by SDS-polyacrylamide gel electrophoresis (4% stacking and 10% separating gels) and then transferred overnight onto nitrocellulose membranes. The membranes were blocked with 1% skim milk in 50 mM Tris-buffered saline containing 0.1% w/v bovine serum albumen (BSA) and 0.01% v/v Triton X-100 and were immunoblotted with polyclonal rabbit antihuman CYP2D antibody[33,35] (A gift from A. Cribb and Merck & Co., Whitehouse Station, NJ) diluted 1:5000. Blots were then incubated with peroxidase-conjugated sheep antirabbit antibody (1:2000, Chemicon international Inc., Temecula, Calif.) and developed with the use of chemiluminescent detection and autoradiography film. Brain stem membranes from saline-treated control rats were serially diluted and used to construct standard curves to determine the linear detection range of detection for the immunoblotting assay. Rat liver microsomes and complementary DNA (cDNA)–expressed rat CYP2D1 (BD Biosciences, Mississauga, Ont.) were used as positive controls. cDNA-expressed rat CYP1A1, CYP1A2, CYP2A1, CYP2A2, CYP2B1, CYP2E1, CYP2C11 and CYP3A2 (BD Biosciences) were used as negative controls. Immunoblots were analyzed with MCID Elite software (InterFocus Imaging Ltd., Linton, UK), and the relative density of each band was expressed as arbitrary density units after background was subracted.

Immunocytochemistry

Brain regions were dissected, fixed in 4% paraformaldehyde and transferred into 20% sucrose in 0.2 M phosphate buffer at 4°C. Frozen sections (12 μm) were collected on silane-coated slides and used for immunocytochemical analysis as previously described.[33] Briefly, sections were blocked in phosphate-buffered saline containing 1% skim milk powder, 1% BSA, 0.1% Triton X-100 and 10% normal horse serum and incubated with anti-CYP2D antibody (1:400 dilution) for 48 hours at 4°C. After incubation with biotinylated antirabbit gamma globulin (Vector Laboratories, Burlington, Ont.), the antigen-antibody complex was visualized by the avidin-biotin complex technique (ABC kit, Vector Laboratories, Burlington, Ont.), followed by reaction with 3,3′-diaminobenzidine and hydrogen peroxide (DAB kit, Vector Laboratories, Burlington, Ont.). Negative control sections were incubated without primary antibody. Sections from nicotine- and saline-treated rats were processed together, and slides were photographed under identical light conditions. Slides were assessed by 2 independent observers. In slides from saline treated animals, the area(s) with lowest staining were assigned a weak (+) designation and the area(s) with highest staining were assigned a strong (+++) designation. All other brain areas from both saline- and nicotine-treated animals were described relative to these regions.

RNA isolation and slot blot hybridization

Total RNA from rat brain samples was isolated with the use of the GenElute Mammalian Total RNA Kit (Sigma, St. Louis, Mo.). RNA slot blot hybridization was performed as previously described.[34] Briefly, brain RNA (0–4 μg) from saline-treated rats was serially diluted to determine the linear region of detection of CYP2D and β-actin RNA for RNA slot blot. Serially diluted human CYP2D6 cDNA (0–48 pg) was used as a positive control and to assess linearity of detection. cDNAs for CYP2A6, CYP2B6, CYP2E1 and CYP3A4 were used as negative controls. Yeast transfer RNA (7.5 μg) was added to all samples and controls to reduce background. Brain RNA (2.5 μg) was applied directly to nylon membranes under vacuum with the use of a Bio-Dot microfiltration apparatus (BioRad, Hercules, Calif.). Membranes were prehybridized and then hybridized with 2 end-labelled rat oligonucleotide probes (each 2.0×10^7 cpm/mL [γ-^{32}P]dATP). The sequences of rat oligonucleotides are 5′-ACCTCATGGATGACAGCATTGGT-3′ and 5′-GCCCTGGGCATCCAGGAAGTGTTC-3′; these are the reverse complementary sequences corresponding to positions 1075 to 1097 and 1261 to 1284 of rat CYP2D1–18 mRNA, respectively. The specific probe for rat β-actin was made as previously described.[34] Blots were washed at room temperature sequentially in 2× standard saline citrate and then 0.5× standard saline citrate with 0.1% sodium dodecyl sulfate (SDS) for 15 minutes each and exposed to Kodak OMAT-XR film for 1–8 days at –80°C. Autoradiography films were analyzed with MCID Elite software.

Data analyses

CYP2D protein levels from immunoblots were expressed as

density units (mean and standard deviation [SD]). The average values were obtained from at least 3 separate experiments. The ratio of CYP2D levels in nicotine-treated animals to those in saline-treated animals was used to express the relative induction of CYP2D. CYP2D mRNA levels were expressed as levels of CYP2D mRNA relative to β-actin mRNA to control for loading efficiency and quality of the RNA. The differences between treatment groups among rat brain regions at 8 hours were tested with the use of 2-way analysis of variance (ANOVA); this was followed by unpaired Student's *t* tests to test differences between nicotine and saline treatments within brain regions. The differences in the ratio of CYP2D mRNA levels in nicotine- to saline-treated animals across time were tested by ANOVA ($p < 0.05$ was considered significant).

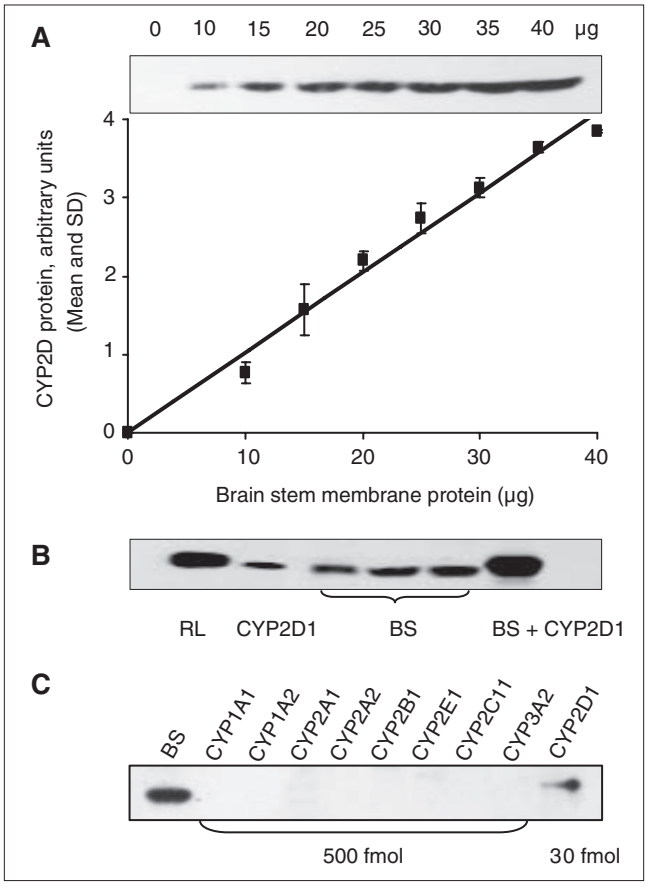

Fig. 1: CYP2D protein detection by immunoblotting in rat brain membranes. A dilution curve of brain stem membrane protein from a saline-treated animal (**A**) shows linear CYP2D protein detection from 10 µg to 40 µg of protein; the insert shows a representative immunoblot. (**B**) CYP2D in brain stem (BS, 15 µg protein) migrates similarly to both rat liver microsomes (RL, 0.4 µg protein) and cDNA-expressed rat CYP2D1 protein (30 fmol), and BS and CYP2D1 comigrate when loaded together in the same well. (**C**) The CYP2D antibody did not cross-react with cDNA-expressed rat CYP1A1, CYP1A2, CYP2A1, CYP2A2, CYP2B1, CYP2E1, CYP2C11 and CYP3A2 but detected a greater than 15-fold lower amount of cDNA-expressed CYP2D1. BS at 15 µg is shown for comparison. SD = standard deviation; cDNA = complementary DNA.

Results

CYP2D in brain

A quantitative immunoblotting assay was developed, and detection of CYP2D in serially diluted brain membrane protein was linear (Fig. 1A). All immunoblots in subsequent experiments were loaded with 15 µg of membrane protein, except for cerebellum, where 13 µg of membrane protein was loaded because of the higher basal CYP2D levels. Brain CYP2D isoforms comigrated with rat liver CYP2D isoforms and cDNA-expressed rat CYP2D1. cDNA-expressed CYP2D1 was added to brain membranes to confirm the detection and comigration (Fig. 1B). No cross-reactivity was observed with other cDNA-expressed rat CYPs, indicating the specificity of polyclonal rabbit antihuman CYP2D antibody under the conditions used in this study (Fig. 1C).

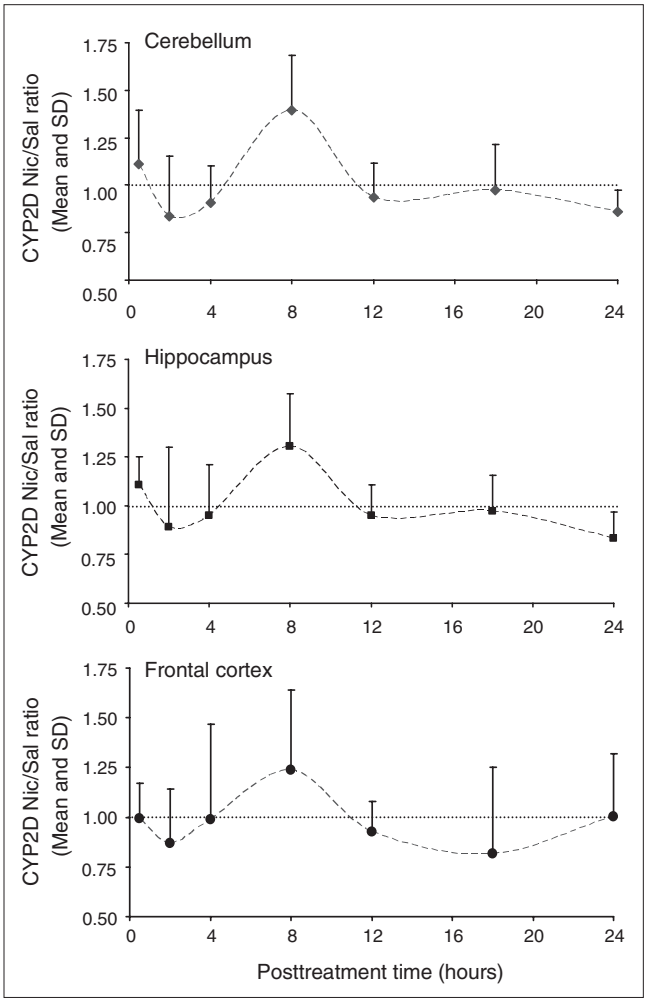

Fig. 2: CYP2D levels peaked at 8 hours after chronic nicotine treatment and returned to control levels by 12 hours after treatment. Data were expressed as the ratio of CYP2D levels in the nicotine group to those in the saline group; $n = 3$ animals per group, except for 8 hours, where $n = 6$ or 7 per group. Nic = nicotine; Sal = saline; SD = standard deviation.

Chronic nicotine induces brain CYP2D maximally at 8 hours

For all 3 regions assessed for CYP2D levels over time, the maximum increase when compared with saline treated controls was seen at 8 hours after the last nicotine treatment; levels returned to saline-treated control values by 12 hours (Fig. 2). To determine whether CYP2D was induced by chronic nicotine treatment in other brain regions, CYP2D protein levels were assessed at 8 hours after the last treatment. CYP2D levels were significantly different among regions ($F_{1,5}$ = 31.93, $p < 0.001$) and between treatments ($F_{1,5}$ = 195.67, $p < 0.001$), and the ANOVA also indicated a significant interaction between treatment and regions ($F_{1,5}$ = 6.41, $p < 0.001$). Compared with the saline-treated control animals, CYP2D levels in rats receiving chronic nicotine treatment were significantly increased by 1.4-fold (t_{12} = 3.41, p = 0.005) in cerebellum, 1.3-fold (t_{11} = 2.60, p = 0.025) in hippocampus, and 3.2-fold (t_{11} = 8.47, $p < 0.001$) in striatum; although not significant, there was some suggestion of increases of 1.25-fold (t_{11} = 1.76, p = 0.11) in brain stem, 1.24-fold (t_{12} = 1.32, p = 0.21) in frontal cortex, and 1.29-fold (t_6 = 1.19, p = 0.28) in thalamus (Fig. 3). At 0.5, 8 and 24 hours after the last injection, no significant induction of hepatic CYP2D was observed from chronic nicotine treatment (98%, 87% and 93% of saline-treated control levels, respectively, data not shown).

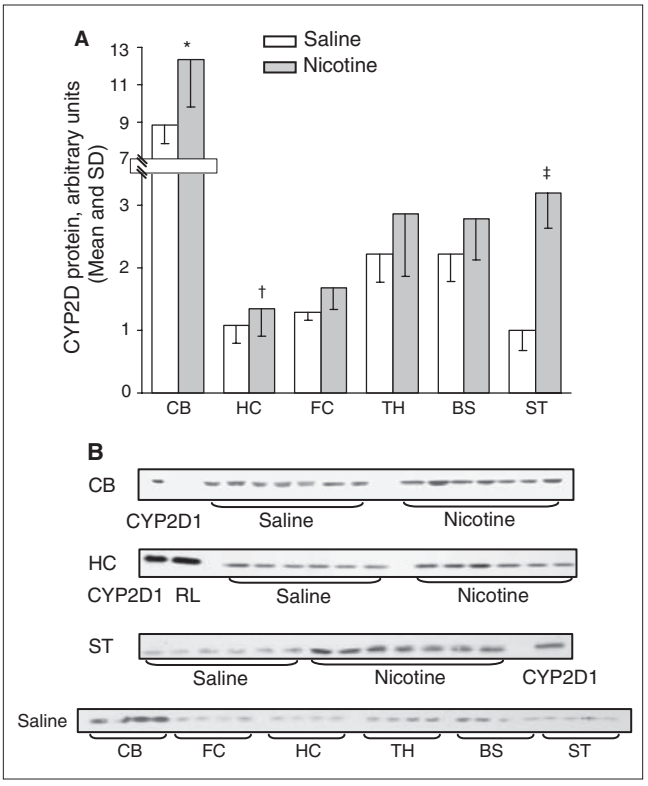

Fig. 3: CYP2D was induced 8 hours after chronic nicotine treatment (**A**) in cerebellum (CB), hippocampus (HC) and striatum (ST) and trended toward an increase in frontal cortex (FC), thalamus (TH) and brain stem (BS). CYP2D levels varied significantly among brain regions in the saline-treated control group (analysis of variance, $p < 0.001$); CYP2D in cerebellum was significantly higher than in all the other regions (post hoc least significant difference $p < 0.001$); n = 6 or 7 per group, *$p < 0.01$, †$p < 0.05$, ‡$p < 0.001$ in comparison with respective saline-treated control animals. The representative immunoblots of CB, HC and ST (**B**) illustrate higher levels in nicotine-treated animals when compared with saline-treated animals.

Cell-specific induction of CYP2D by chronic nicotine treatment

The intensity and cellular distribution of CYP2D immuno staining varied among the brain regions of saline-treated control animals (Table 1), with strongest staining seen in cerebellum. In general, the intensity of immunostaining was consistent with the level of immunoreactivity detected by immunoblotting. Compared with the saline-treated animals, CYP2D immunostaining in the nicotine-treated animals was more intense in caudate-putamen, globus pallidus and nucleus accumbens (Table 1, Fig. 4A, B, C, D). In cerebellum, neurons in the granular cell layer and neurons and astrocytes in the white matter stained more strongly in nicotine-treated animals (Table 1, Fig. 4E, F). In hippocampus, neurons in the granular layer of the dentate gyrus and pyramidal cells in CA1 and CA2 were more intensely stained in nicotine-treated animals (Table 1, Fig. 4G, H, J, K). In frontal cortex, pyramidal cells in layer II-VI in the nicotine-treated animals stained slightly more strongly than in the saline-treated animals. No immunostaining was detected in control sections incubated without primary antibody (Fig. 4I).

Table 1: Brain CYP2D protein immunocytochemical staining in saline- and nicotine-treated rats

Brain region	Saline	Nicotine
Frontal cortex		
Layer I	++	++
Layer II-VI, pyramidal cells	++	+++
White matter*	+	+
Hippocampus		
Dentate gyrus, granule cells	++	+++
CA1, CA2, CA3		
Polymorphic layer	++	++
Pyramidal cells	+	+/++
Molecular layer	++	++
Striatum		
Caudate	++	++++
Globus pallidus	++	+++
Nucleus accumbens	++	++++
Anterior nucleus	++	++
Thalamic nuclei	++	++
Subthalamic nuclei	+	+
Substantia nigra		
Pars compacta	++	++
Pars reticulata	++	++
Cerebellum		
Molecular layer	+++	++++
Purkinje cells	+	+
Granular layer	+++	++++
White matter*	++	++++

++++ = very strong; +++ = strong; ++ = moderate; + = weak.
*Neurons and glial cells.

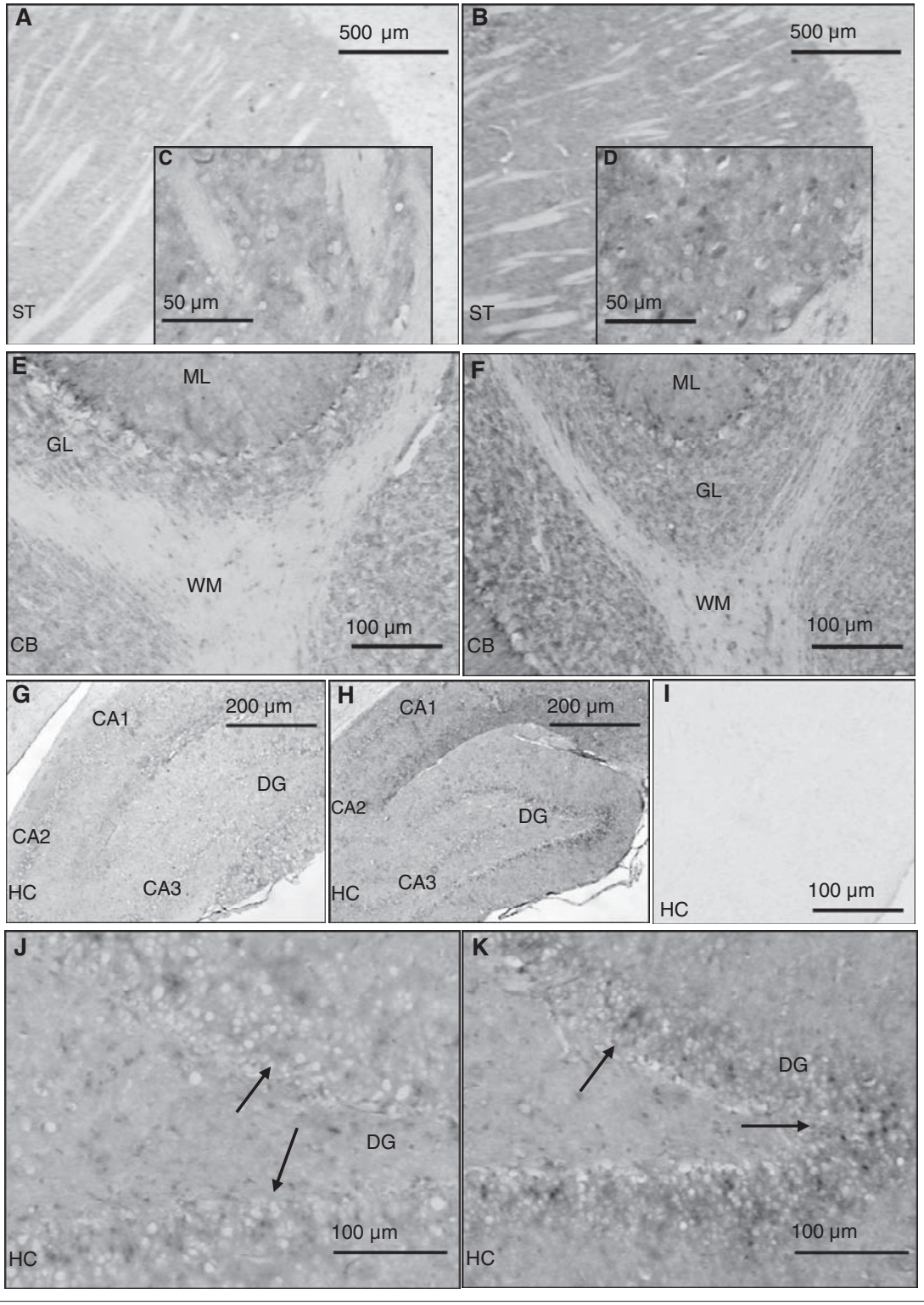

Fig. 4: CYP2D immunostaining is more intense in striatum, cerebellum and hippocampus of nicotine-treated rats when compared with saline-treated control rats at 8 hours after treatment. Staining was stronger in neurons and neuropil in striatum (**A, B, C, D**). In cerebellum (**E, F**), staining was similar in the molecular layer (ML), stronger in neurons in the granular cell layer (GL) and in neurons and glial cells in the white matter (WM). In hippocampus (**G, H, J, K**), staining was stronger in the dentate gyrus granular cells (DG, arrows) and in the pyramidal cells of regions CA1–CA3. There was no immunostaining of control sections without primary antibody (**I**). ST = striatum; CB = cerebellum; HC = hippocampus.

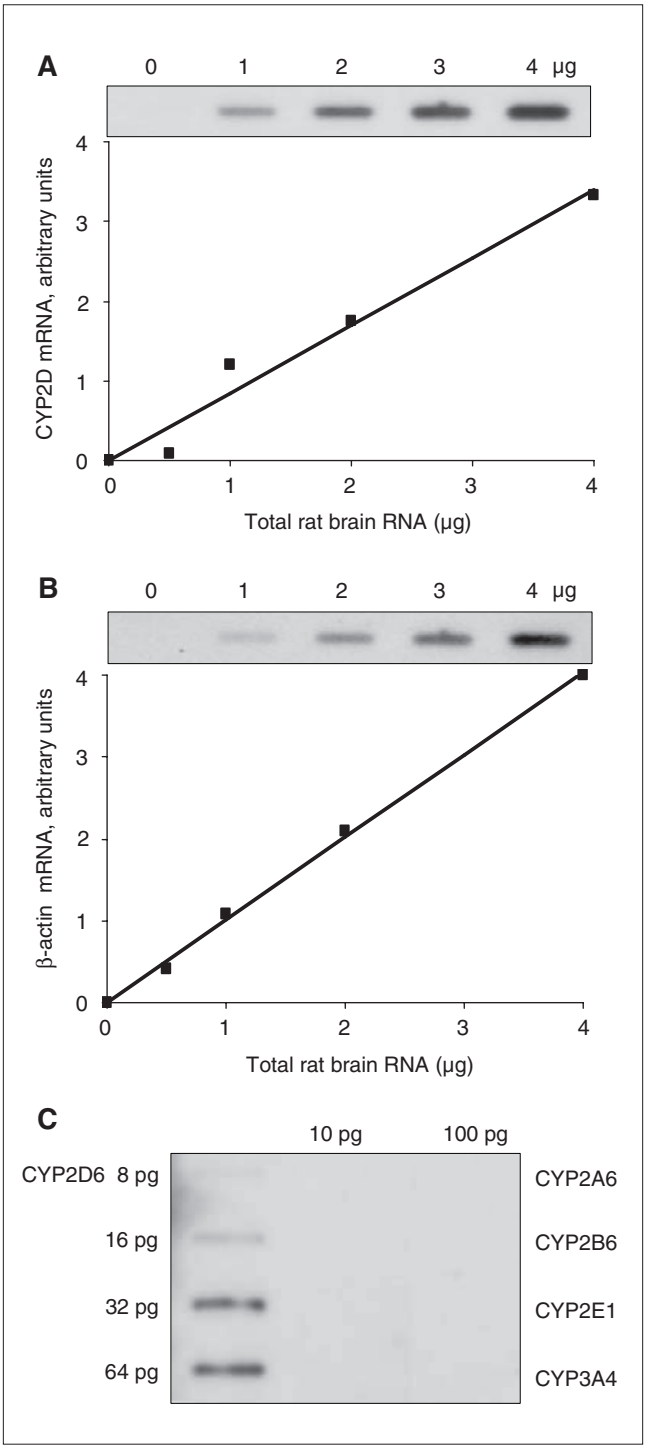

Fig. 5: CYP2D mRNA was detected in rat brain by slot blotting. (**A**) Detection of CYP2D in a serial dilution of saline-treated rat hippocampus RNA was linear; the insert shows a representative immunoblot. (**B**) Detection of β-actin mRNA in a serial dilution of saline-treated rat hippocampus RNA was linear; the insert shows a representative immunoblot. (**C**) There was no detection of CYP2A6, CYP2B6, CYP2E1 or CYP3A4 cDNAs loaded at 10 pg and 100 pg, but CYP2D6 cDNA was detected as low as 16 pg. mRNA = messenger RNA; cDNA = complementary DNA.

No change in mRNA levels after chronic nicotine treatment

Detection was linear for CYP2D and β-actin mRNA in serially diluted total RNA from hippocampus of saline-treated control animals and for serially diluted CYP2D6 cDNA (Fig. 5A, B, C). No signal was detected for cDNAs of CYP2A6, CYP2B6, CYP2E1 and CYP3A4 at 10 pg and 100 pg, while CYP2D6 cDNA was easily detected at 16 pg (Fig. 5C). CYP2D mRNA levels were expressed relative to β-actin levels. There was no significant difference across time in the ratio of nicotine- to saline-treated CYP2D mRNA values for either hippocampus ($F_{5,10}$ = 0.13, p = 0.98) or cerebellum ($F_{4,8}$ = 0.97, p = 0.47) (Fig. 6A, B).

No induction by acute treatment at 8 hours

At 8 hours after a single nicotine injection, we observed no induction of CYP2D in cerebellum (t_4 = 2.78, p = 0.79), hippocampus (t_5 = 2.57, p = 0.58) or striatum (t_4 = 2.78, p = 0.65) (Fig. 7).

Discussion

This is the first demonstration that chronic nicotine can induce CYP2D enzymes in rat brain. This implies that the higher CYP2D levels observed in human smokers in comparsion

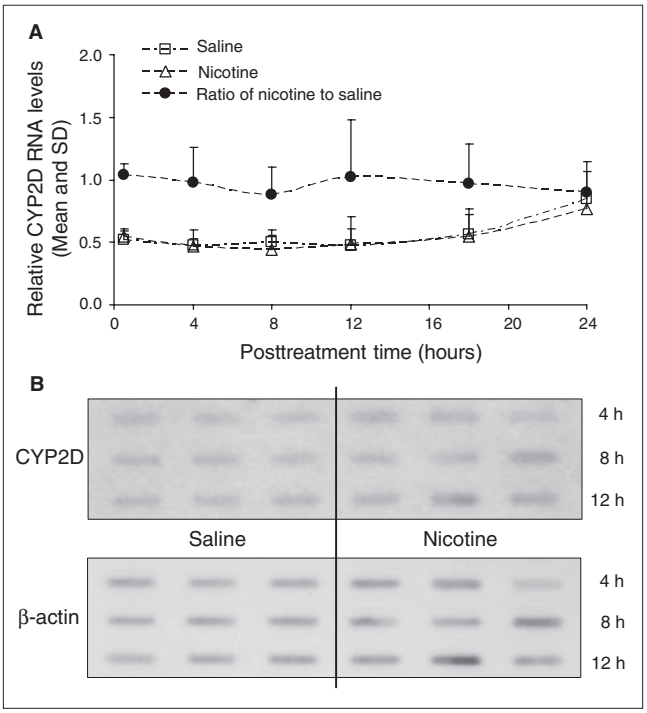

Fig. 6: Brain CYP2D mRNA was not induced by chronic nicotine treatment. A posttreatment time course of relative CYP2D/β-actin mRNA levels in the saline-treated and nicotine-treated groups and the ratio of nicotine-treated to saline-treated groups shows no significant differences at any time tested (**A**). A representative mRNA slot blot (**B**) shows brain CYP2D and β-actin at 4 hours, 8 hours and 12 hours in the saline- and nicotine-treated groups (n = 3 per group, in rows). SD = standard deviation; mRNA = messenger RNA.

with nonsmokers[26] is due at least in part to the nicotine in tobacco smoke. Nicotine is widely used as a smoking-cessation treatment, mainly in the form of a patch or gum, and is in trials as a therapy for conditions such as ulcerative colitis[36] and neurodegenerative diseases such as Alzheimer disease.[37] In humans, smoking does not alter CYP2D hepatic activity,[27] and, in this study, we observed that chronic nicotine does not alter rat hepatic CYP2D levels. This suggests that not only active and passive smokers but also people exposed to therapeutic nicotine may have increased brain levels of CYP2D while their hepatic levels remain unchanged.

We have previously demonstrated that CYP2D is enzymatically functional in rat brain and that the protein levels detected by immunoblotting correlate with this activity. In this study, immunocytochemistry showed that the higher brain CYP2D levels in nicotine-treated rats, quantified by immunoblotting, were localized to specific cells that could cause increased CYP2D-mediated metabolism in the local microenvironment. These increases may affect individuals exposed to the commonly used drug nicotine in terms of their response to clinical centrally acting drugs metabolized by CYP2D, their susceptibility to neurotoxins either activated or inactivated by CYP2D and the general homeostasis of endogenous neurochemicals

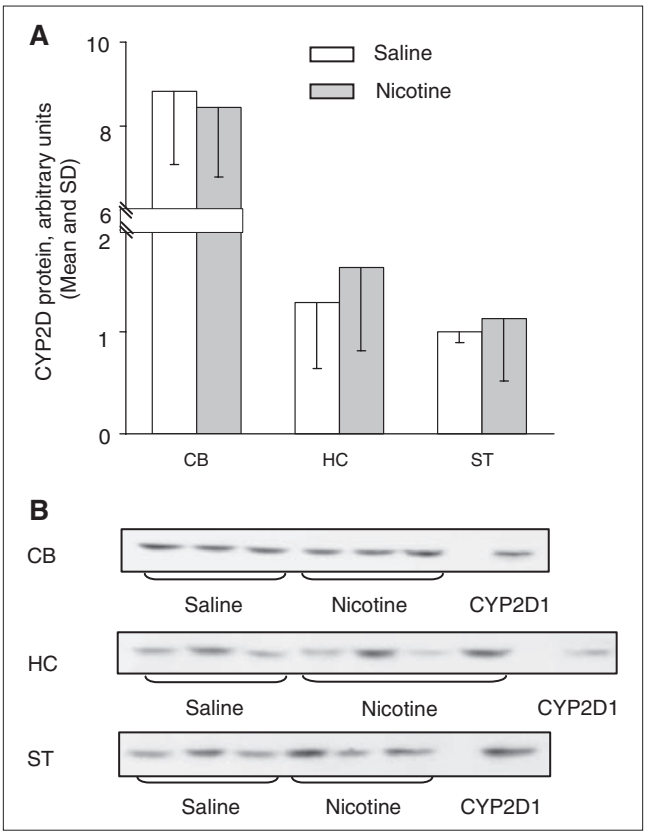

Fig. 7: (**A**) There was no induction of brain CYP2D levels 8 hours after a single acute nicotine treatment in cerebellum (CB), hippocampus (HC) or striatum (ST); *n* = 3 or 4 per group, and the average values were obtained from 4 separate experiments. (**B**) The representative immunoblots illustrate no changes between saline and nicotine treatments in the regions tested. SD = standard deviation.

metabolized by CYP2D. For example, there is a dose-dependent negative association of smoking with Parkinson disease such that smokers are at lower risk for Parkinson disease.[38,39] CYP2D6 can inactivate many of the neurotoxins that can cause symptoms of Parkinson disease[12,13]; therefore, neuroprotection against this disease by smoking may be due in part to nicotine-induced brain CYP2D6-mediated neurotoxin inactivation. Although smoking is clearly hazardous, it may be possible to use therapeutic nicotine to provide some degree of protection from Parkinson disease. These data suggest that there are drugs and xenobiotics, such as nicotine and alcohol[19,26] and compounds not yet identified, that can increase human brain CYP2D levels without changing hepatic CYP2D activity.

The dosage of nicotine used in this study resulted in plasma nicotine levels that rapidly peaked to a maximum of 202 ng/mL by 30 minutes and were undetectable at 4 hours posttreatment.[34,40] The mean plasma levels were 70 ng/mL, comparable to levels of 20–50 ng/mL seen in smokers.[41] Because of the route (subcutaneous) and bolus nature of nicotine delivery, peak levels in the brain may have been higher than the peak plasma levels. In rats, however, the half-life of nicotine in brain is thought to be shorter (50 min)[42] than the half-life in plasma (150 min).[43] This suggests that nicotine levels decline in both brain and plasma before brain CYP2D levels peak. According to our findings, the induction of rat brain CYP2D by a single daily nicotine injection for 7 days is best characterized as short-lasting, with a delay of several hours between the peak of CYP2D induction in brain regions tested and the peaks in either plasma nicotine concentration or estimated brain nicotine concentration. Conversely, a single dose of nicotine failed to induce brain CYP2D, suggesting that the effect is additive over time or that repeated exposure is needed to trigger activation of the mechanism. Under continuous infusion of nicotine over time, where plasma and brain nicotine levels remain at a steady state, this induction pattern may be different and may be longer lasting. To avoid withdrawal symptoms, human smokers maintain their plasma nicotine levels by repeated intake of nicotine; these levels remain steady throughout the day and decline during overnight abstinence. This suggests that induction of CYP2D6 in human brain by smoking would be long-lasting, owing to prolonged (20 h) exposure to nicotine, which is consistent with observed elevated levels of CYP2D6 in human brains from smokers after various autopsy times.[26] From our observations in rats, human brain CYP2D levels would most likely return to normal after smoking cessation.

There were some similarities in the patterns of CYP2D in the brains of human smokers and in the brains of nicotine-treated rats; for example, there were higher levels in the globus pallidus of both species. However, there were also some differences; for example, in human smokers, higher levels of CYP2D were found in the Purkinje cells of the cerebellum and the pigmented neurons of the substantia nigra,[19,26] whereas no changes in CYP2D were observed in these cells or regions in nicotine-treated rats. This could be a species difference in regulation of CYP2D isozymes, or it could be due to a difference in drug dosage and mode of delivery, or it could reflect the different effects of nicotine alone and nicotine

combined with the other components of cigarette smoke. In addition, human smokers may differ from nonsmokers on various measures not assessed, such as dietary differences.

Induction of brain CYP2D by nicotine may be through the induction of a specific isoform in brain that is not expressed in liver, such as CYP2D18,[44] or through the induction of an isoform usually expressed at high levels in liver but at low levels in brain, such as CYP2D2.[45] Whereas nicotine induced rat brain CYP2D protein levels, there were no detected changes in mRNA levels — a finding similar to the induction of rat brain CYP2D by clozapine and ethanol.[46,47] This indicates that the increase in brain CYP2D by nicotine is probably posttranscriptional by increased translation efficiency or protein stabilization, perhaps by inhibiting the ubiquitination and degradation of brain CYP2D. However, because the results by RNA slot blot provided an overview of total CYP2D mRNA, we cannot rule out the possibility of small changes in mRNA for one or more specific isoforms of the CYP2D subfamily. The solvent toluene can induce CYP2D4, an isoform expressed primarily in brain[46] with or without activation of mRNA expression, indicating that both transcriptional and posttranscriptional regulation can occur.[46,48] Chronic nicotine treatment has also been shown to induce brain CYP2E1 protein levels without an increase in mRNA levels when rats were treated with the same regimen used in the present study, suggesting posttranscriptional regulation of CYP2E1 by nicotine.[32] The precise mechanism by which nicotine induces brain CYP2D requires further elucidation.

In conclusion, we have demonstrated that nicotine, a widely used drug, can induce brain CYP2D levels by means of a nontranscriptional mechanism that does not alter hepatic levels. This suggests that individuals exposed to nicotine through smoking or through nicotine replacement therapy may have increased CYP2D-mediated brain metabolism of centrally acting drugs, neurotoxins and endogenous neurochemicals. The animal model in this study will be useful in establishing the metabolic role of centrally expressed CYPs and the effects of altered central expression levels of CYPs on therapeutic response, drug interactions and risk of neurotoxicity.

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   Krüger et al
   J Psychiatry Neurosci 2006;31(4):263-270
2. **Treatment of primary insomnia with melatonin: a double-blind, placebo-controlled, crossover study**
   Almeida Montes et al
   J Psychiatry Neurosci 2003;28(3):191-196
3. **The long-term impact of treatment with electroconvulsive therapy on discrete memory systems in patients with bipolar disorder**
   MacQueen et al
   J Psychiatry Neurosci 2007;32(4):241-249
4. **Efficacy of escitalopram in the treatment of major depressive disorder compared with conventional selective serotonin reuptake inhibitors and venlafaxine XR: a meta-analysis**
   Kennedy et al
   J Psychiatry Neurosci 2006;31(2):122-131
5. **Etiology of infantile autism: a review of recent advances in genetic and neurobiological research**
   Trottier et al
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6. **Use of the Medication Event Monitoring System to estimate medication compliance in patients with schizophrenia**
   Diaz et al
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7. **Empirical evaluation of language disorder in schizophrenia**
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   J Psychiatry Neurosci 2007;32(4):250-258
8. **Platelet serotonin levels support depression scores for women with postpartum depression**
   Maurer-Spurej et al
   J Psychiatry Neurosci 2007;32(1):23-29
9. **Use and abuse of over-the-counter analgesic agents**
   Abbott et al
   J Psychiatry Neurosci 1998;23(1):13-34
10. **Monoamine oxidase-A polymorphisms might modify the association between the dopamine D2 receptor gene and alcohol dependence**
    Huang et al
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