

### 2011 CCNP Heinz Lehmann Award paper

# Cytochrome P450-mediated drug metabolism in the brain

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Cytochrome P450 enzymes (CYPs) metabolize many drugs that act on the central nervous system (CNS), such as antidepressants and antipsychotics; drugs of abuse; endogenous neurochemicals, such as serotonin and dopamine; neurotoxins; and carcinogens. This takes place primarily in the liver, but metabolism can also occur in extrahepatic organs, including the brain. This is important for CNS-acting drugs, as variation in brain CYP-mediated metabolism may be a contributing factor when plasma levels do not predict drug response. This review summarizes the characterization of CYPs in the brain, using examples from the CYP2 subfamily, and discusses sources of variation in brain CYP levels and metabolism. Some recent experiments are described that demonstrate how changes in brain CYP metabolism can influence drug response, toxicity and drug-induced behaviours. Advancing knowledge of brain CYP-mediated metabolism may help us understand why patients respond differently to drugs used in psychiatry and predict risk for psychiatric disorders, including neurodegenerative diseases and substance abuse.

## Introduction

Cytochrome P450 enzymes (CYPs) are found throughout the animal and plant kingdoms and are responsible for the oxidative metabolism of a wide variety of both exogenous and endogenous compounds. A large proportion of therapeutic drugs acting on the central nervous system (CNS) are metabolized by CYPs, primarily by members of the CYP2 family, to either active or inactive metabolites.<sup>1,2</sup> For example, CYP2D6 activates codeine to morphine, inactivates the antidepressant desipramine and converts the active antipsychotic parent drug risperidone to an equally active metabolite.<sup>3-5</sup> There is a great deal of variation in individual responses to centrally acting drugs, and in some cases the plasma levels of these drugs and/or their metabolites do not predict their therapeutic effect.<sup>6</sup> Drug metabolism by CYPs takes place primarily in the liver, but CYP enzymes are also found in many other tissues, including brain.<sup>7</sup> It is predicted that local brain metabolism of centrally acting drugs at their site of action can influence their

therapeutic efficacy independently of liver metabolism, and differences in brain levels of CYP enzymes can contribute to the observed interindividual variation in drug response.<sup>7,8</sup>

There are many isoforms of CYPs, and these are classified into 18 families and 57 subfamilies based on their amino acid identity.<sup>9</sup> Members of families 1, 2 and 3 primarily metabolize xenobiotics, such as drugs and environmentally derived compounds, and some endogenous substrates, whereas other vertebrate CYP family members primarily metabolize endogenous compounds.<sup>5,7,10-12</sup> The CYP2 family metabolizes a large proportion of CNS-acting pharmacologics, such as antidepressants and antipsychotics; drugs of abuse, such as amphetamine and ethanol; and some endogenous neurochemicals, such as dopamine and serotonin. Some examples are presented in Tables 1 and 2.<sup>5,13-19</sup> As in the liver, the expression levels of brain CYP forms vary greatly among individuals. This can be caused by factors that affect both brain and liver CYP expression levels, including genetic variation, which can result in differences in the plasma levels of drugs and their

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*J Psychiatry Neurosci* 2013;38(3):152-63

Submitted July 16, 2012; Revised Oct. 22, 2012; Accepted Oct. 25, 2012.

DOI: 10.1503/jpn.120133

metabolites.<sup>1,3,20,21</sup> About 10% of white people have gene variants that result in a functionally deficient CYP2D6 enzyme, and these poor metabolizers often respond inadequately to drugs, such as codeine, that are activated by CYP2D6.<sup>5</sup> The frequency of genetic variants of *CYPs* differs among ethnic groups, and, in conjunction with environmentally induced variation in brain CYP levels (e.g., from different diets, herbal medications), this may contribute to the differences seen in response to centrally acting drugs among different ethnic populations.<sup>21–23</sup> Many CYPs are also sensitive to induction by xenobiotics, and there are a number of drugs and environmental compounds that are organ-specific inducers.<sup>24</sup> Brain CYPs are often regulated quite differently from the hepatic forms; for example, brain CYP2B6 is elevated in smokers, whereas liver CYP2B6 is unaffected by smoking.<sup>25</sup> As a result, smokers may respond differently than nonsmokers to a CNS-acting drug or neurotoxin that is a CYP2B6 substrate, without any differences in drug or neurotoxin plasma levels between the 2 groups.

Other factors can also alter CYP levels in the brain but not in the liver; for example, we are just beginning to understand how the pattern of expression of brain CYPs changes with age. In humans, the brain CYP2D6 enzyme level is low at birth, increases gradually with age, and is highest in adults older than 65 years,<sup>26</sup> whereas hepatic CYPs increase quickly after birth to adult levels and remain constant with age.<sup>27,28</sup> This suggests that for some centrally acting drugs, older adults may respond differently than younger adults. These differences may contribute to the observation that individuals older than 75 years respond less than younger patients to desipramine, a drug that is inactivated by CYP2D6.<sup>29</sup>

## CYPs in the brain

The first reports of CYP-like metabolic activity in the brain appeared in the literature in the mid-1970s,<sup>30,31</sup> and in 1977, Sasame and colleagues<sup>32</sup> were the first to show that CYP and nicotinamide adenine dinucleotide phosphate reductase activity were present in the rat brain. During the next decade, researchers continued to quantify brain CYPs and to assess their metabolic activity toward different substrates.<sup>33–38</sup> Most brain CYPs metabolize substrates with similar affinities, and

relative substrate and inhibitor selectivity, to their hepatic counterparts. However, there are CYP forms that are unique to the brain or that are much more highly expressed in brain than in other tissues, including CYP2D4 and CYP2D18<sup>39–41</sup> and some members of the CYP3A families in rats.<sup>42</sup> In addition, the subcellular localization of brain CYPs is often different from hepatic CYPs, partly owing to the differences in cellular structure between hepatocytes and neurons. However, while hepatic CYPs are expressed primarily in the endoplasmic reticulum, brain activity is also found in the mitochondrial and plasma membrane fraction<sup>37</sup> and other cell membrane compartments.<sup>37,43</sup> There are some forms specific to the mitochondria, such as the NH<sub>2</sub>-terminal-cleaved CYP1A1,<sup>44</sup> and some CYPs are found in neuronal dendrites that lack the endoplasmic reticulum.<sup>25,45,46</sup> In the 1990s, there was a massive expansion of our knowledge on the expression of different brain CYP families and isoforms and their substrate specificities,<sup>46–50</sup> primarily owing to improved analytical and immunological techniques. In concert with the ability to study extrahepatic CYP enzymes, there was a growing interest in their localized function not only in brain tissues, but also in others, such as intestine and lung tissues.

Early investigations estimated that CYPs are expressed at levels almost 100 times lower in the brain than in the liver,<sup>38</sup> and it was difficult to conceive how these brain CYPs could have any functional consequences. However, as CYP protein expression was mapped across brain regions and cell types, it became evident that their expression levels vary greatly among specific brain regions and that, at the cellular level, expression can be as high as levels in hepatocytes.<sup>51</sup> These enzymes are found in both neurons and glial cells, in the cell bodies and throughout the cell processes. Some isozymes, such as CYP1A1, CYP2B, CYP2E1 and CYP3A, are predominantly found in neurons,<sup>45,52–55</sup> whereas others are found in both neurons and glial cells. For example, CYP2B is found in astrocytes in areas with high densities of neuronal fibre tracts, in the end-feet of astrocytes surrounding cerebral blood vessels and in pyramidal neurons of the frontal cortex.<sup>25,51,56</sup> CYP2D is found in both neurons and glial cells as well as in areas of the brain that are not protected by the blood-brain barrier, such as the choroid plexus.<sup>43,46,57</sup> In humans, CYP1B1 is one of the main CYPs found in cerebral

**Table 1: Examples of central nervous system-acting substrates for 3 drug-metabolizing cytochromes P450<sup>5,13–18</sup>**

Enzyme	CNS-acting drugs	Endogenous	Other drugs and toxins
CYP2B6	Bupropion, diazepam, ketamine, methadone, meperidine, nicotine, pentobarbital, phencyclidine, propofol, sertraline, selegiline, tramadol	17-β estradiol, anandamide, arachidonic acid, estrone, serotonin, testosterone	3,4-methylenedioxy-amphetamine (ecstasy), chlorpyrifos, cyclophosphamide, DEET, efavirenz, ifosfamide, malathion, paraquat, parathion
CYP2D6	Amyltriptyline, brofaromine, clomipramine, codeine, citalopram, clozapine, desipramine, dextromethorphan, ethylmorphine, fluoxetine, fluvoxamine, haloperidol, hydrocodone, imipramine, mianserin, mirtazapine, nicergoline, nortryptaline, oxycodone, paroxetine, perphenazine, risperidone, tramadol, tranylcypromine, venlafaxine, zuclopenthixol	5-methoxytryptamine, anandamide, progesterone, tyramine	MPTP, parathion, tamoxifen
CYP2E1	Enflurane, felbamate, halothane, isoflurane, sevoflurane, trimethadione	17-β estradiol, arachidonic acid, estrone, prostaglandin	Acetaminophen, acetone, aniline, benzene, carbon tetrachloride, chloroform, chlozoxazone, ethanol, NNK, phenol, theophylline, trichloroethane

CNS = central nervous system; DEET = N,N-diethyl-m-toluamide; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

microvessels at the blood–brain interface, where it may act in conjunction with transporters, such as ATP-binding cassette transporters, to regulate passage of xenobiotics in and/or out of the brain.<sup>8,58–60</sup> The interaction of a variety of transporters, in addition to local CYP-mediated metabolism, may play an important role in regulating the levels of centrally acting drugs in the brain and, thereby, their therapeutic effects. Brain CYPs are inducible, and the organ- and brain-region specificity of induction was found to be quite complex.<sup>7,24,43,45,61</sup> This, with the progressive discoveries that brain CYPs can metabolize endogenous neurochemicals, such as tyramine to dopamine by CYP2D6<sup>62,63</sup> and testosterone to 16 $\alpha$ -hydroxytestosterone by CYP2B6,<sup>10,64</sup> lent credence to the idea that these enzymes may play a functional role in the brain in both endogenous and exogenous pathways.

## Endogenous brain CYP function

The complexity of CYP expression and regulation is likely related to the multitude of functions of these enzymes throughout the body, including within the brain. There is a growing list of endogenous substrates of CYPs that are converted to neurally active metabolites; Table 2 gives examples for some CYP2 family isozymes.<sup>13–15,19</sup> Some of the earliest indications that brain CYPs may have important neurologic endogenous functions came from personality testing, where individuals with a CYP2D6 poor metabolizer genotype and phenotype were observed to have different personality traits than CYP2D6 normal metabolizers.<sup>65</sup> Several studies of this type

**Table 2: Examples of central nervous system–acting endogenous substrates of cytochromes P450<sup>13–15,19</sup>**

Enzyme	Substrate	Metabolite
CYP1A1	Melatonin, estradiol, arachidonic acid, progesterone, all-trans-retinal	6-hydroxymelatonin, 2-hydroxyestradiol, 16–19 HETE, 16 $\beta$ -hydroxyprogesterone, retinol, all-trans-retinoic acid
CYP1B1	Melatonin, estradiol	6-hydroxymelatonin, 2-hydroxyestradiol
CYP2B	Arachidonic acid, testosterone, serotonin, anandamide, all-trans-retinoic acid	20,19 HETE, 16 $\alpha$ -hydroxytestosterone, hydroxylamine, nitric oxide, 11,12-EET-EA, 4-hydroxyretinoic acid, 4-oxoretinoic acid
CYP2C	Testosterone, progesterone, arachidonic acid, serotonin, harmaline, harmine, linoleic acid, melatonin, all-trans-retinoic acid	11 $\beta$ -hydroxytestosterone, 21-hydroxyprogesterone, EETs, 19 HETE, hydroxylamine, nitric oxide, harmalol, harmol, 11-hydroxyoctadecadienoic acid, 6-hydroxyserotonin, N-acetylserotonin, 18-hydroxyretinoic acid
CYP2D	5-methoxytryptamine, octopamine, synephrine, tyramine, progesterone, anandamide, harmaline, harmine	Serotonin, noradrenaline, adrenaline, dopamine, 11-deoxycorticosterone, 16 $\alpha$ -, 16 $\beta$ -, 17 $\beta$ -, 2 $\beta$ -, 6 $\beta$ -hydroxyprogesterone, 20-HETE-EA, harmalol, harmol
CYP2E1	Arachidonic acid, linoleic acid, oleic acid	18,19-HETE, hydroxylinoic acid, 17-, 18-hydroxyoleic acid

EA = ethanolamide; EET = epoxyicosatrienoic acids; HETE = hydroxyicosatetraenoic acid.

have shown an effect of genetic variability in CYP2D6 on personality,<sup>66–68</sup> and recently this has been linked more closely to the brain in reports where different CYP2D6 genotypes were found to be associated with different resting brain perfusion rates<sup>69</sup> and with different levels of brain activation during a cognitive task.<sup>70</sup> Similar associations of genotype and personality or mood have been observed for CYP2C19, CYP2E1 and CYP19 (aromatase).<sup>71–74</sup> These enzymes are expressed in the brain, and they can all metabolize endogenous neural substrates, suggesting that these genotype–phenotype–personality associations may be manifested, at least in part, through altered brain metabolism of CNS neurochemicals. CYP2D6 can participate in the metabolism of neurochemicals that influence psychological state, such as the formation of the catecholamines dopamine from tyramine<sup>62,63,75</sup> and serotonin from 5-methoxytryptamine<sup>76,77</sup> and the metabolism of the endogenous cannabinoid anandamide<sup>78</sup> and the neurosteroid progesterone.<sup>79</sup> CYP2C19 can metabolize serotonin to hydroxylamine<sup>80</sup> as well as the sex hormones testosterone, progesterone and estradiol that are known to affect brain function and personality traits, such as aggression.<sup>81,82</sup> CYP2E1 can metabolize the fatty acid neural signalling molecule arachidonic acid, which is abundant in the brain and is required for neurologic health,<sup>83</sup> and CYP19 aromatase can metabolize testosterone to estradiol, both of which can influence personality traits, including aggression, impulsivity and anxiety.<sup>81,82</sup> This ever-growing list of neurochemicals that can be metabolized by CYPs raises the questions of whether changes or differences in brain levels of specific CYPs can cause subtle shifts in the neurochemical homeostasis in the brain, what the consequences of these shifts may be, and how we can assess them experimentally and clinically.

## Demonstration of brain CYP activity in vivo

Although these earlier studies of the associations of CYP genotype with personality traits were highly suggestive of an endogenous function of brain CYPs, it is only over the last few years that persuasive evidence of brain CYPs' function in vivo has emerged. Research on the function of brain CYPs has been technically challenging for several reasons. In the intact organism, CYPs in the liver produce many of the same metabolites as those in the brain, and many of these can cross the blood–brain barrier from the periphery to the brain, making it difficult to separate the relative contributions of hepatic from brain metabolism in vivo. In addition, CYP expression levels in the brain are very low, and brain CYPs are highly labile when studied in vitro. Rat brain CYP2D activity is extremely sensitive to freezing: more than 40% of enzymatic function is lost from rat brain membranes after 7 days of frozen storage, and more than 80% of function is lost when membranes are stored in Tris buffer instead of artificial cerebrospinal fluid (ACSF).<sup>84</sup> In spite of this, there have been many in vitro studies demonstrating that brain membranes can metabolize a variety of substrates, including drugs,<sup>85–87</sup> toxins<sup>88,89</sup> and endogenous neurochemicals.<sup>62,90</sup> However, it was not clear if in vivo there were adequate cofactors or appropriate membrane environments in the brain for these enzymes to

be functional toward these substrates. For example, *ex vivo* studies on CYP1A1 demonstrated that brain cytoplasmic heme levels were rate-limiting to catalytic activity and also affected the membrane insertion of the protein, suggesting that brain CYP enzymes *in vivo*, particularly if induced, may not always have sufficient available heme to be functional.<sup>91,92</sup>

Some convincing evidence that brain CYPs can function *in vivo* arose from the use of brain slices to demonstrate the activity of CYPs in brain tissue *ex vivo* without additional heme, cofactors or energy sources. Sagittal sections of mouse brain were used to demonstrate a possible role for CYPs in neurotoxicity of the Parkinsonsonian symptom-inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).<sup>93</sup> Mice were treated with either phenobarbital, a drug that induces many of both brain and liver CYPs, or pargyline, a drug that reduces levels of many brain CYPs. Brain slices were prepared from treated mice, exposed to MPTP and assessed for neurotoxicity. Compared with slices from control mice, induction of brain CYPs by phenobarbital resulted in increased toxicity, and reduction of brain CYPs by pargyline resulted in decreased toxicity. This suggests a role for brain CYPs in MPTP-induced toxicity, at least *ex vivo*. A later, similar *ex vivo* study found that neurotoxicity from MPP<sup>+</sup> (a neurotoxic metabolite of MPTP) was also increased in brain slices from phenobarbital-treated mice and decreased in brain slices pretreated with CYP inhibitor.<sup>94</sup> This study suggested that CYPs may be involved in the metabolic activation of this neurotoxin in the brain slices *ex vivo*. More recent studies have used rat hippocampal slices to demonstrate that the synthesis of estradiol and testosterone in the slices *ex vivo* was abolished by pretreatment with CYP inhibitors, suggesting that the synthesis of these compounds may be at least in part CYP-mediated in the brain.<sup>95,96</sup> Another strategy that was developed to study the kinetics of drug disposition in the brain *in vivo* was the whole-head perfusion preparation.<sup>97-100</sup> These preparations delivered perfusate through the carotid artery, collected perfusate from the venous system, and were used to investigate the brain distribution and elimination of a number of drugs, including imipramine, desipramine, propranolol, diclofenac and clonidine.<sup>98,101</sup> In 1 study, the levels of 5-hydroxytryptamine recovered in the perfusate were higher after infusion of tranylcypromine, a monoamine oxidase inhibitor, and the levels were further increased after infusion of tryptophan.<sup>100</sup> This preparation showed potential for investigating metabolism in the brain, at least of biogenic amines such as serotonin, but unfortunately it has not been developed further or used to investigate brain metabolism of other substrates.

A recent study using microdialysis investigated the CYP2D-mediated formation of dopamine from tyramine in the striatum of a living rat, with and without CYP2D inhibition.<sup>63</sup> This study showed that there are sufficient levels of metabolism in the brain to be detected by microdialysis and that this technique may prove to be a useful tool to investigate *in vivo* brain CYP-mediated metabolism of not only endogenous neurochemicals, but also of drugs and neurotoxins. Injection of selective CYP inhibitors into the brain has been used to investigate the mechanism of action of the nonopioid analgesic im-

progan, and these studies showed that brain CYP epoxygenase activity is required for imiprogran's analgesic effects.<sup>102</sup>

The *in situ* enzymatic activity of brain CYP2B in a living animal was recently demonstrated with a technique that uses the CYP-mediated metabolism of a mechanism-based (also known as suicide) inhibitor to its reactive metabolite.<sup>103</sup> Mechanism-based inhibitors are substrates of the enzyme that are converted to metabolites that bind covalently to the enzyme, thereby inactivating it irreversibly. These experiments used tritiated 8-methoxypsoralen (8-MOP), a mechanism-based inhibitor of CYP2B,<sup>104</sup> which is enzymatically converted by CYP2B to a radiolabelled dihydrodiol metabolite. This metabolite reacts covalently with CYP2B apoprotein,<sup>104</sup> causing the functional CYP2B enzyme to become both radiolabelled and inactivated. It is important to note that the CYP2B will only become radiolabelled if it is enzymatically functional. Radioactive 8-MOP was microinjected into the left frontal cortex of an anaesthetized, living rat. CYP2B was then immunoprecipitated with a specific monoclonal antibody raised against CYP2B from membranes prepared from the frontal cortex tissue, and the amount of radiolabelled CYP2B was assessed. In additional experiments to ensure the CYP2B-specificity of this enzymatic reaction, 1 side of the frontal cortex was microinjected with C-8-xanthate, another specific mechanism-based inhibitor of CYP2B,<sup>105</sup> before injection into both sides of the frontal cortex with radiolabelled 8-MOP. Pretreatment of 1 side of the frontal cortex with C-8-xanthate significantly reduced the amount of CYP2B that became radiolabelled by 8-MOP compared with the other side of the frontal cortex, providing further evidence that brain CYP2B was enzymatically functional *in vivo*<sup>103</sup> (Appendix 1, Fig. S1, available at [cma.ca/jpn](http://cma.ca/jpn)).

To test whether induced brain CYP2B is also functional, this experiment was repeated in rats that had received 7 daily subcutaneous injections of 1 mg/kg of nicotine, a regimen previously shown to increase CYP2B protein in the frontal cortex by approximately 2-fold.<sup>51</sup> The amount of radiolabelled CYP2B protein immunoprecipitated from the frontal cortex of nicotine-treated rats was twice that immunoprecipitated from saline-treated rats,<sup>103</sup> indicating that nicotine-induced cortical CYP2B is also functional (Appendix 1, Fig. S1).

Further proof-of-principle experiments using intracerebroventricular (ICV) delivery of radioactive 8-MOP confirmed that induced CYP2B was functional.<sup>106,107</sup> They also demonstrated that CYP2B is active throughout the brain, and that this mechanism-based inhibitor reaches all parts of the brain after ICV injection, from olfactory bulbs in the anterior to the brainstem in the posterior of the brain.<sup>107</sup> Radioactive CYP2B was detected in increasing amounts over time and was found at higher levels at 24 hours compared with 4 hours after ICV, with no radioactivity detected in the liver at either time point.<sup>106,107</sup> These experiments confirmed that after ICV injection, CYP2B enzymatic activity was inhibited throughout the brain, and that this inhibition persisted for at least 24 hours. These data provided the parameters for inhibiting and inducing CYP2B activity throughout the brain in a living animal, paving the way for studies demonstrating a role for CNS-specific CYP enzymatic activity in drug response.

## Consequences of altered brain CYP activity

These recent confirmations that brain CYPs are active *in vivo* and the validation of inhibitor and inducer approaches to manipulate brain CYP levels and enzyme activity have facilitated the development of animal models to demonstrate the behavioural consequences of alterations in brain CYP activity. These models can also be used to investigate the roles of brain CYP-mediated metabolism in areas such as the efficacy of centrally acting drugs, neurotoxicity and drug dependence.

### Drug efficacy

The responses to drugs acting on the CNS are not always directly related to drug and metabolite levels circulating in the plasma.<sup>6</sup> One explanation for this apparent disconnect may be local brain drug metabolism. A rat model was used to investigate local brain metabolism of propofol, a commonly used anesthetic that varies considerably in its effect among individuals.<sup>108</sup> In animal studies, the sedation from propofol correlates more strongly with the brain levels than with the plasma levels of this drug.<sup>109</sup> The parent drug propofol is metabolically inactivated by CYP2B to an inactive hydroxide and also by uridine 5'-diphospho-glucuronosyltransferases through glucuronidation.<sup>110,111</sup> A rat model was used to investigate the potential role of brain CYP2B-mediated metabolism of propofol in the response to this anesthetic. After an intraperitoneal injection of propofol, rats slept for a period of time that was proportional to the brain levels of propofol, but sleep time had no correlation with plasma propofol levels.<sup>107</sup> In this model, rats were treated with nicotine to induce brain, but not hepatic, CYP2B.<sup>51</sup> Compared with saline-treated controls, nicotine-treated rats slept on average for 60% less time.<sup>107</sup> They also had lower brain levels of propofol than saline-treated controls (Appendix 1, Fig. S2), likely owing to faster metabolism of propofol after induction of brain CYP2B. In a complementary set of experiments, brain CYP2B was inactivated by ICV injection of the CYP2B-specific mechanism-based inhibitor C-8-xanthate. Rats that received ICV inhibitor slept on average 4 times longer than controls treated with ICV ACSF, and this increase in sleep time was proportional to the dose of ICV C-8-xanthate (Appendix 1, Fig. S2). Brain propofol levels were higher in rats that received ICV inhibitor than in ACSF controls (Appendix 1, Fig. S2), likely owing to slower metabolic elimination of propofol within the brain. In contrast, neither plasma propofol levels nor hepatic CYP2B *ex vivo* enzyme activity were altered in ICV C-8-xanthate rats compared to ACSF controls (Appendix 1, Fig. S2). Inhibition of brain CYP2B was also able to reverse the nicotine-induced reduction in sleep time, providing further evidence that induced brain CYPs are functional. These studies demonstrate that the central effects of a drug can be influenced by its metabolism in the brain, underscoring the importance of understanding what factors can influence brain levels of CYP drug-metabolizing enzymes. Brain CYP2B6 levels are higher in smokers than case controls, whereas hepatic CYP2B6 levels do not differ.<sup>25,112</sup> Interestingly case reports have suggested that human smokers require higher doses of propofol to

achieve anesthesia and experience less postanesthesia side effects than nonsmokers.<sup>113,114</sup> This is consistent with a role for elevated brain CYP2B6 seen in smokers, resulting in faster inactivation of propofol in the brain.

### Neurotoxicity

Parkinson disease is a progressive neurodegenerative disease, whose etiology is not entirely understood. Most cases are sporadic, and exposure to environmental or endogenous toxins, with or without a genetic predisposition, is thought to play a large role in these cases.<sup>115</sup> There is evidence that individuals with a *CYP2D6* poor metabolizer genotype are at greater risk for this disease and that this risk is even greater with exposure to neurotoxic pesticides.<sup>116,117</sup> *CYP2D6* metabolizes and inactivates a number of compounds that can cause parkinsonian symptoms, including MPTP, parathion, isoquinoline and  $\beta$ -carbolines.<sup>5,12,18</sup> The impaired ability of *CYP2D6* poor metabolizers to inactivate these or similarly structured compounds may contribute to their increased risk for Parkinson disease. In contrast to *CYP2D6* poor metabolizers, smokers are at lower risk for Parkinson disease,<sup>118</sup> and nicotine is neuroprotective in several neurotoxin-induced animal models of the disease.<sup>119</sup> In humans, *CYP2D6* expression levels are higher in the brains of smokers than nonsmokers,<sup>61,120</sup> and in rats and monkeys, brain CYP2D is higher after chronic nicotine treatment.<sup>61,121</sup> This suggests that genetically decreased levels of brain CYP2D6 can reduce local inactivation of neurotoxins that can cause Parkinson disease, while induction of brain CYP2D6 (e.g., by nicotine or smoking) may increase local inactivation, reducing an individual's relative risk for the disease. This is supported by *in vitro* laboratory studies using MPTP, a Parkinson-causing compound, and its neurotoxic metabolite MPP<sup>+</sup>, both of which can be inactivated by CYP2D6.<sup>122</sup> Overexpression of CYP2D6 in PC12 cells (from rat adrenal medulla) was protective against neurotoxicity from MPP<sup>+</sup>,<sup>123</sup> whereas inhibition of CYP2D6 in neuronal SHY5Y cells (from human neuroblastoma) increased MPTP and MPP<sup>+</sup> neurotoxicity.<sup>122</sup> *CYP2D6* is expressed in human brain regions affected by Parkinson disease, such as the substantia nigra. A post mortem study of brains of individuals with Parkinson disease showed that *CYP2D6* extensive metabolizers had about 50% lower brain levels of *CYP2D6* than their age-matched, nondiseased controls.<sup>26</sup> This reduction was seen in various regions, including those unaffected by Parkinson disease, such as the cerebellum and hippocampus, supporting the hypothesis that lower brain *CYP2D6* levels seen in individuals with Parkinson disease may be a predisposing factor. Together, these data support a contributing role for lower brain *CYP2D6* in the increased risk for Parkinson disease, likely through modulation of local neurotoxin metabolism.

The role of brain CYP2B-mediated metabolism in the neurotoxicity of chlorpyrifos has also been investigated. Exposure to this commonly used organophosphate pesticide can cause cognitive defects and other neurologic effects in humans.<sup>124</sup> Chlorpyrifos is converted to chlorpyrifos oxon primarily by CYP2B, and this oxon metabolite is responsible for

neurotoxicity through the inhibition of acetylcholinesterase (AChE).<sup>125,126</sup> Chlorpyrifos oxon is quickly inactivated in both the liver and blood and therefore is unlikely to reach the brain, suggesting a role for local brain CYP2B-mediated activation of chlorpyrifos to the oxon in the neurotoxicity.<sup>127</sup> In rats, an acute exposure to chlorpyrifos results in well-characterized neurochemical and behavioural symptoms of toxicity.<sup>128</sup> A recent study used this rat model to investigate the effects of inhibiting brain CYP2B activity on chlorpyrifos neurotoxicity.<sup>129</sup> Decreasing CYP2B brain activity should decrease local chlorpyrifos oxon production and attenuate the neurotoxic effects of chlorpyrifos in this model. Compared with ACSF-treated controls, rats that received ICV C-8-xanthate, a selective mechanism-based irreversible inhibitor of CYP2B, had higher brain levels of chlorpyrifos, lower brain levels of chlorpyrifos oxon and reduced inhibition of brain AChE consistent with reduced behavioural indicators of chlorpyrifos neurotoxicity, such as abnormal gait, incline plane slippage and righting reflex latency (Appendix 1, Fig. S3). Inhibitor-treated rats had less hypothermia, another early sign of chlorpyrifos toxicity.<sup>130</sup> Consistent with these reductions in toxicity being mediated by inhibition of brain, rather than peripheral, activation to the chlorpyrifos oxon, there were no differences in plasma chlorpyrifos levels or AChE inhibition (Appendix 1, Fig. S3). These data strongly suggest that brain CYP2B-mediated chlorpyrifos metabolic activation plays a role in the resulting neurotoxicity. These data not only support a role for brain CYPs in neurotoxicity, but also raise the possibility of developing the use of CYP inhibitors, which can cross the blood-brain barrier or can be delivered to the brain, as a treatment for acute chlorpyrifos poisoning.

### Drug dependence

Smoking is a substantial health problem, and yet even with improved public education and smoking cessation treatments, 17% of Canadians are smokers.<sup>131</sup> It is important, therefore, to fully understand the biological basis of tobacco dependence. Nicotine is the main component of cigarette smoke that causes tobacco dependence, and genetic variation in the main hepatic nicotine-metabolizing enzyme CYP2A6 can affect several smoking behaviours.<sup>132</sup> Genetic variation in CYP2B6 can also influence smoking; CYP2B6 slow metabolizers progress to tobacco dependence more quickly and have more difficulty quitting than normal metabolizers.<sup>133,134</sup> This enzyme does not contribute to hepatic nicotine metabolism or circulating plasma levels of nicotine, but it is present in the brain, where it may metabolize nicotine and other endogenous substrates, such as serotonin and neurosteroids.<sup>64,80</sup> There are a number of animal models of nicotine dependence and withdrawal that can be used to investigate the role of brain CYP2B in these smoking behaviours.

Nicotine dependence is seen in both human smokers and animals, and abrupt cessation of exposure to nicotine results in withdrawal symptoms. In a rat model of smoking withdrawal,<sup>135</sup> nicotine (6 mg/kg/d) was delivered by constant infusion from a subcutaneously implanted minipump for 7 days. The pump was then removed, and the stereotypic be-

haviours associated with spontaneous withdrawal were quantified and compared between rats that received a continuous infusion of nicotine and those that received saline.<sup>136</sup> Rats that received nicotine typically exhibited withdrawal behaviours for up to 24 hours after pump removal.<sup>135</sup>

This withdrawal paradigm was used in conjunction with continuous brain CYP2B inhibition through ICV infusion of C-8-xanthate from a minipump to investigate the role of brain CYP2B-mediated nicotine metabolism in nicotine withdrawal.<sup>137</sup> Inhibition of brain CYP2B was continued throughout the withdrawal assessments. Control rats receiving ACSF displayed typical nicotine withdrawal signs during the first 24 hours. Rats receiving C-8-xanthate did not display withdrawal signs until 3 days after pump removal, and they displayed more withdrawal signs overall than ICV ACSF controls (Appendix 1, Fig. S4). The intensity of withdrawal assessed by the number of stereotypic behaviours depended on the dose of ICV-delivered C-8-xanthate, with higher doses producing more withdrawal. However, the delay in appearance of withdrawal signs was constant for the doses of C-8-xanthate tested. These changes in intensity and timing of withdrawal were not driven by changes in hepatic nicotine metabolism, as plasma nicotine and cotinine levels and ex vivo hepatic nicotine metabolism were not different between rats treated with ICV C-8-xanthate and those treated with ICV ACSF. There were no differences in CYP2B enzyme levels in either brain or liver tissues between rats treated with ICV C-8-xanthate and those treated with ICV ACSF. The exact mechanism whereby reduced brain CYP2B-mediated nicotine metabolism increases and delays withdrawal in this model is unclear. Further investigation is required to determine whether processes such as acetylcholine receptor adaptation are involved or whether this is mediated by brain nicotine metabolites other than cotinine.

In a well-validated rat model of smoking,<sup>138</sup> rats received an intravenous infusion of nicotine directly into the jugular vein when they pressed a lever in an operant chamber. This model can also be used in conjunction with inhibition of brain CYP2B-mediated nicotine metabolism to investigate the role of brain CYP2B in acquisition of nicotine dependence. Preliminary data suggest that reducing brain CYP2B activity increases the rewarding properties of nicotine.<sup>139</sup> As these studies progress, they promise some important insights into the role of brain CYP2B metabolism in influencing nicotine's rewarding properties and consequently smoking behaviour, possibly by altering brain levels of nicotine and its metabolites.

Including the studies described above, evidence is mounting in support of a role of brain CYP-mediated metabolism in response to drugs that act on the CNS, risk for neurotoxicity and neurologic disease, and general healthy brain homeostasis. It is therefore important to gain a better understanding of the factors that can influence brain CYP levels and activity, and the mechanisms by which changes can occur.

### Regulation of brain CYPs

Regulation of brain CYPs is varied and complex. Regulation is not only organ-specific, but also inducer- and species-specific

and brain region- and cell-specific. This complexity can be illustrated by the inducing properties of 2 commonly used CNS-acting drugs, nicotine and ethanol. Depending on the CYP examined, these are potent inducers of hepatic and/or brain CYPs.

### *Organ specificity*

Organ specificity is seen in all species, including rats, monkeys and humans. In rats and monkeys, nicotine treatment does not alter hepatic CYP2D protein levels, but does increase brain CYP2D protein levels<sup>61,121</sup> (Appendix 1, Fig. S5). Similarly, human smokers, compared with nonsmokers, have similar hepatic levels but higher brain CYP2D levels<sup>46,61,140</sup> (Appendix 1, Fig. S5). In contrast, ethanol induces CYP2B in the rat liver, but not in the rat brain.<sup>141</sup>

### *Inducer specificity*

Different drugs or inducers also have different effects on individual brain CYPs; for example, in rats, brain CYP2B is induced by nicotine but not ethanol treatment.<sup>51,141</sup>

### *Brain region and species specificity*

The pattern of induction among brain regions is also complex and varies with inducer, species and CYP isozyme. Nicotine and ethanol have different regional effects on brain CYP2E1. This is illustrated in the rat brain, where CYP2E1 is induced in the olfactory bulbs, frontal cortex and cerebellum by both nicotine and ethanol; in contrast, nicotine, but not ethanol, induces CYP2E1 in the olfactory tubercle and brain stem, and ethanol, but not nicotine, induces CYP2E1 in the hippocampus<sup>45</sup> (Appendix 1, Fig. S6). When we look across species, an inducer can affect the same isozyme, but in different brain regions. In rats, nicotine induces CYP2B in several brain regions, including the frontal cortex, olfactory bulbs and tubercle, striatum and brain stem; however, in monkeys, nicotine induction of CYP2B has only been reported for the frontal cortex<sup>51,142</sup> (Appendix 1, Fig. S7). In addition to inducer and species differences in the pattern of induction among brain regions, a single inducer within a single brain region can affect different CYP isozymes in different ways. For example, in rats nicotine induces CYP2B and CYP2E1, but not CYP2D, in the frontal cortex and brainstem; nicotine induces CYP2D, but not CYP2B or CYP2E1, in the hippocampus; and nicotine induces CYP2D and CYP2E1, but not CYP2B, in the cerebellum<sup>45,51,121</sup> (Appendix 1, Fig. S8).

### *Cell specificity*

Regulation of brain CYPs becomes even more complex when examined at the cellular level. Induction is seen in specific cells within a brain region; for example, nicotine induces rat CYP2B primarily in the pyramidal cells of the frontal cortex layers III–VI.<sup>51</sup> Cellular induction can be inducer-specific. For example, both ethanol and nicotine induce CYP2E1 in rat cerebellum; however, at the cellular level ethanol, but not

nicotine, induces CYP2E1 in the cerebellar granule cell layer, and nicotine, but not ethanol, induces CYP2E1 in glial cells in the cerebellar white matter.<sup>45</sup> Similarly, nicotine induces CYP2E1 in the cerebellum in both rats and monkeys; however, this induction is seen in the cerebellar Purkinje cells of monkeys, but not of rats.<sup>45,143</sup>

Although species and inducer differences are likely to exist, these comparisons should be made with some caution, particularly with reference to human data. Not only can the administration route, dose and duration of exposure to an inducer differ among animal models, but for human data, sample numbers are often quite small, and many other unknown variables also exist. For example, compared with nonsmokers, smokers consume more nicotine but also many other tobacco compounds; they also may be more likely to consume alcohol.

There is a variety of xenobiotics, in addition to nicotine and ethanol, that regulate brain CYP levels, and many of these are also CYP substrates. The antipsychotic clozapine can induce CYP2D in the rat brain, specifically in neurons of the substantia nigra, ventral tegmental area, olfactory bulbs, brain stem and Purkinje and granule cells in the cerebellum.<sup>144,145</sup> The antipsychotic thioridazine increases CYP2D activity in the striatum and cerebellum, but reduces CYP2D activity in the substantia nigra and nucleus accumbens.<sup>144</sup> The antiepileptic phenytoin induces CYP2B and CYP3A enzymes in the mouse brain, which results in altered brain testosterone metabolism.<sup>64,146</sup> This suggests that some drugs may induce or repress their own local metabolism in the brain (e.g., clozapine is inactivated by CYP2D enzymes), and chronic treatment may therefore affect central drug levels and resulting drug efficacy. Since many drugs are coadministered, this could also potentially result in drug interactions (e.g., induction of brain CYP2D by clozapine could result in faster CYP2D-mediated metabolic inactivation of some substrate antidepressants or increased activation of the oral opiate codeine to morphine). In addition, some of these centrally acting drugs may modify CYP-mediated brain metabolism of endogenous neurochemicals, such as the metabolism of dopamine by CYP2D6 and the metabolism of neurosteroids by CYP3A, which in turn may modify therapeutic outcome.

## **Mechanisms of brain CYP induction**

Although there is extensive reporting on the complex patterns of induction of brain CYP proteins, much less is known of the underlying mechanisms. For example, the induction of CYP2B by phenobarbital and the induction of CYP2E1 by ethanol have been studied extensively in the liver, but the regulation of these isozymes in the brain is less well understood. As seen for patterns of CYP induction, the reported mechanisms of induction also show organ, species and isozyme specificity. In rats, toluene induces CYP2D protein in both the liver and brain; however, RNA levels are increased in the liver but remain unchanged in the brain,<sup>145</sup> suggesting 2 different molecular mechanisms of action of a chemical on the same isozyme. Phenobarbital treatment increases both CYP2B RNA and protein in both the rat liver

and brain; however, a longer treatment period may be required to increase CYP2B RNA in the brain than in the liver (4 v. 1–2 d, respectively).<sup>147</sup> In contrast, in rabbits, phenobarbital treatment increases CYP2B RNA and protein in the liver, but neither RNA nor protein are affected in the brain.<sup>148</sup> Like phenobarbital, nicotine treatment increases CYP2B RNA and protein in the rat brain, suggesting transcriptional regulation,<sup>51</sup> but nicotine increases CYP2D and CYP2E1 protein only, with no accompanying increase in RNA.<sup>45,121</sup>

The expression or induction of many CYPs is regulated by nuclear receptors, such as the arylhydrocarbon receptor (AhR), constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor (PPAR), pregnane X receptor (PXR) and retinoid X receptor (RXR). For example, in the liver, AhR regulates CYP1A1, CYP1A2 and CYP1B1; CAR regulates CYP1A1, CYP1A2, CYP2Bs and CYP3As; PPAR regulates CYP2Cs; and PXR regulates CYP2A6, CYP2B, CYP2C, CYP3A and CYP4F families.<sup>149–152</sup> The expression levels of these nuclear receptors vary among tissues. For example, CAR and PXR are expressed highly in the liver, but at low levels in the brain, whereas PPAR $\beta$  and  $\delta$ , RXR $\beta$  and AhR may be expressed more highly in the brain than in the liver.<sup>153,154</sup> The expression of these nuclear receptors also varies among brain regions. For example, CAR was detectable only in the human caudate nucleus;<sup>155</sup> PXR was expressed at high levels in the human thalamus, pons and medulla,<sup>156</sup> PPAR and RXR were expressed highly in the rat brainstem but at low levels in the substantia nigra,<sup>157</sup> and AhR was expressed highly in the rat olfactory cortex but at low levels in the amygdala.<sup>154</sup> In the brain, nuclear receptor expression is also cell-specific. For example, in the cerebellum Golgi cells express all PPAR subtypes, whereas Purkinje cells express only PPAR $\beta$ , and Golgi cells express RXR $\alpha$ ,  $\beta$  and  $\gamma$ , but granule cells express only RXR $\alpha$ .<sup>157</sup> The arylhydrocarbon receptor is expressed highly in CA3 pyramidal cells and moderately in cerebellar granule cells, but is not expressed in Purkinje cells.<sup>154</sup> Much remains to be clarified in terms of the expression of these transcription factors within the brain and their role in regulating CYPs. In addition to the differential expression of nuclear receptors among organs, brain regions and cell types, these receptors are genetically variable; they exist as organ-specific splice variants; their expression can be modulated by other receptors, such as the estrogen receptor; and there is cross-talk among nuclear receptors.<sup>155,156,158–161</sup>

The previous examples illustrate that diverse and distinct mechanisms likely underlie the specificity of CYP expression and regulation among organs, tissues or brain regions. A mechanism that may allow for a variety of forms of CYPs is alternative mRNA splicing, and this is seen widely in the brain.<sup>162–164</sup> For example, splice variants of CYP1A1 have been found in the brain that differ from those found in other organs. However, the impact on metabolic activity of these variations is still relatively unclear.<sup>33,165</sup> Other mechanisms may include variation in nuclear receptors, altered phosphorylation and glycosylation states, posttranslational modifications, microRNA and epigenetic control of expression.<sup>166–168</sup> Differential regulation of CYP expression may also occur through indirect effects, such as altered hormonal levels.<sup>169,170</sup>

## Conclusion

A better understanding of the factors, such as age, genotype and chronic exposure to common inducers like nicotine and ethanol, that can alter brain CYPs and their metabolic activity will likely be most important in understanding interindividual differences in response to drugs acting on the brain. In addition, further exploration of the metabolism of endogenous neurochemicals by brain CYPs may increase our understanding of variations in mood, aggression, personality disorders and other psychiatric conditions. Similarly, investigating the role of brain CYP metabolism in neurotoxicity may also contribute to our understanding of the underlying mechanisms of diseases such as Parkinson disease and to the risk of such diseases developing. Understanding how and exactly where brain CYPs function may eventually facilitate the development of novel therapies, such as targeting local CNS activation of anticancer drugs. Knowledge of CYP and transporter function in the blood–brain barrier may increase our ability to deliver active forms of drugs specifically to the brain. Although there have been substantial advances in knowledge of brain CYPs, much remains to be explored, particularly for a better understanding of individual response to drugs acting on the CNS as well as relative risk for neurotoxicity or brain disease.

**Acknowledgements:** The authors were supported by the Centre for Addiction and Mental Health; the Centre for Addiction and Mental Health Foundation; the National Institute on Drug Abuse (R21DA029160); the Canadian Institutes of Health Research (MOP97751); a Canada Research Chair in Pharmacogenetics to R.F. Tyndale.

**Competing interests:** As above. R.F. Tyndale also declares travel support from the Canadian College of Neuropsychopharmacology, having consulted for McNeil and Novartis, institutional support from the National Institutes of Health (NIH) and the Canadian Institutes of Health Research, honoraria and travel support for lectures from several universities and scientific societies, book royalties and payment from the NIH for educational presentations.

**Contributors:** Both authors contributed to review design, data acquisition and analysis, article writing and review, and both approved publication.

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