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Short Communication

Cytochrome P450 mRNA Expression in the Rodent Brain: Species-, Sex-, and Region-Dependent Differences[S]

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ABSTRACT

Cytochrome P450 (P450) enzymes play a critical role in the activation and detoxication of many neurotoxic chemicals. Although research has largely focused on P450-mediated metabolism in the liver, emerging evidence suggests that brain P450s influence neurotoxicity by modulating local metabolite levels. As a first step toward better understanding the relative role of brain P450s in determining neurotoxic outcome, we characterized mRNA expression of specific P450 isoforms in the rodent brain. Adult mice (male and female) and rats (male) were treated with vehicle, phenobarbital, or dexamethasone. Transcripts for CYP2B, CYP3A, CYP1A2, and the orphan CYP4X1 and CYP2S1 were quantified in the liver, hippocampus, cortex, and cerebellum by quantitative (real-time) polymerase chain reaction. These P450s were all detected in the liver with the exception of CYP4X1, which was detected in rat but not mouse liver. P450 expression profiles in the brain varied regionally. With the exception of the hippocampus, there were no sex differences in regional brain P450 expression profiles in mice; however, there were marked species differences. In the liver, phenobarbital induced CYP2B expression in both species. Dexamethasone induced hepatic CYP2B and CYP3A in mice but not rats. In contrast, brain P450s did not respond to these classic hepatic P450 inducers. Our findings demonstrate that P450 mRNA expression in the brain varies by region, regional brain P450 profiles vary between species, and their induction varies from that of hepatic P450s. These novel data will be useful for designing mechanistic studies to examine the relative role of P450-mediated brain metabolism in neurotoxicity.

Introduction

The cytochrome P450 (P450) superfamily is a diverse group of enzymes that catalyze the oxidative metabolism of not only endogenous substrates but also xenobiotics, including environmental contaminants of significant public health concern that target the nervous system, including polychlorinated biphenyls, polybrominated diphenyl ethers, and organophosphorus pesticides (Ariyoshi et al., 1995; Foxenberg et al., 2007; Erratico et al., 2013; Feo et al., 2013). Biotransformation of these compounds by P450s can result in bioactivation or detoxication, and the balance between these activities influences the bioeffective dose, and thus the neurotoxic outcome, following environmental exposures, as shown in studies of humans and animal models (Foxenberg et al., 2007; Curran et al., 2011; Kim et al., 2011; Crane et al., 2012; Khokhar and Tyndale, 2012).

Much of the research effort to characterize P450-mediated metabolism of neurotoxic compounds has focused on the liver. However, it is now evident that P450s are expressed in a number of extrahepatic tissues, including brain (Ding and Kaminsky, 2003; Ferguson and Tyndale, 2011). Although total P450 content in the human and rodent brain is generally significantly lower than that in the liver (Warner et al., 1988; Bhamre et al., 1992; Volk et al., 1995), recent evidence from rat studies demonstrates that P450-mediated metabolism in the brain can contribute significantly to neurotoxicity (Khokhar and Tyndale, 2012; Zhou et al., 2013). These data coupled with reports that P450 expression in the brain may vary between anatomic regions of the brain (Warner et al., 1988; Dutheil et al., 2009) have led to growing interest in the putative role of brain P450s in determining sensitivity and response to neurotoxic compounds via modulation of local metabolite levels (Meyer et al., 2007; Ferguson and Tyndale, 2011; Ravindranath and Strobel, 2013).

Rodents are important models for studying the relative influence of brain versus liver P450s on neurotoxicity; however, most of our knowledge of P450 expression in the rodent brain is derived from studies of whole brain homogenates, and there is a paucity of data on regional P450 expression in the rodent brain. Additional questions include whether the well known sex- and species-specific differences in hepatic P450 expression extend to the brain, and whether P450s in the brain respond to classic inducers of hepatic P450 expression. Here, we address these questions by comparing P450 transcript levels in three distinct regions of the rodent brain relative to expression levels in the liver under basal conditions and following treatment with phenobarbital and dexamethasone, which are classic inducers of hepatic CYP2B and CYP3A expression (reviewed by Corcos and Berthou, 2008; Greenblatt et al., 2008). We also assessed the influence of sex and species on P450 expression profiles in the brain using the male mouse as the reference. We studied CYP2B (mouse CYP2B10/rat CYP2B1/2), CYP3A (mouse CYP3A11/rat CYP3A2), and CYP1A2 because these isoforms have been implicated in the metabolism of polychlorinated biphenyls (Kania-Korwel et al., 2008, 2012; Curran et al., 2011), polybrominated diphenyl ethers (Erratico et al., 2013; Feo et al., 2013), and organophosphorus pesticides (Tang et al., 2001; Foxenberg et al., 2007). Two orphan P450s, CYP4X1 and CYP2S1 (Guengerich et al., 2010), were also included in this study because

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ABBREVIATIONS: CI, confidence interval; CO, corn oil; DEX, dexamethasone; P450, cytochrome P450; PB, phenobarbital.

CYP2S1 is abundantly expressed in many extrahepatic tissues (Choudhary et al., 2003) and CYP4X1 is predominantly expressed in the rodent brain (Bylund et al., 2002; Al-Anizy et al., 2006). The data reported herein demonstrate brain region–specific expression of P450s in the rodent brain that is sex- and species-dependent and generally not altered by the classic inducers phenobarbital and dexamethasone under conditions that significantly induce P450 expression in the liver.

Materials and Methods

Animals and Treatments. Experiments involving animals were approved by the Institutional Animal Care and Use Committee at the University of Iowa. Male and female C57BL/6 mice (7–8 weeks) were obtained from the Jackson Laboratory (Bar Harbor, ME) and randomly assigned to one of four groups: 1) phenobarbital (PB; Sigma-Aldrich, St. Louis, MO) at 102 mg/kg/day in saline or 2) saline at 20 ml/kg/day, i.p., for 3 consecutive days; 3) dexamethasone (DEX; Sigma-Aldrich) at 50 mg/kg/day in corn oil (CO) or 4) CO (Fisher Scientific, Pittsburg, PA) at 10 ml/kg/day, i.p., for 4 consecutive days (Kania-Korwel et al., 2008). Male Sprague-Dawley rats (8 weeks) were purchased from Harlan, Inc. (Indianapolis, IN), acclimated for 1 week, then randomly assigned to one of four groups: 1) PB at 102 mg/kg/day in saline or 2) saline at 5 ml/kg/day, i.p., for 3 consecutive days; 3) DEX at 50 mg/kg/day in CO or 4) CO vehicle control at 5 ml/kg/day, i.p., for 4 consecutive days (Kania-Korwel et al., 2008). Animals were euthanized 24 hours after the last treatment by CO_2 asphyxiation followed by cervical dislocation. Brain regions and livers were immediately collected on ice, weighed, placed in RNALater (Qiagen, Valencia, CA) overnight, and then stored at −80°C. The effects of treatments on liver and total body weight are summarized in Supplemental Tables 1–3.

Assessment of mRNA Levels by Quantitative Polymerase Chain Reaction. Tissue levels of isoform-specific P450 transcripts were quantified using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). P450 mRNA levels were normalized to the reference gene phosphoglycerate kinase 1, and relative expression ratios between treated and vehicle control animals were calculated by the Pfaffl method (Pfaffl, 2001) using REST 2009 software (Qiagen, Valencia, CA). Statistical analysis was performed using the built-in randomization techniques of REST 2009 (detailed descriptions of RNA isolation and mRNA quantification and analyses are provided in the Supplemental Material; Supplemental Tables 6 and 7 list primer sequences and amplification efficiencies of primers sets, respectively).

Results and Discussion

Adult male C57BL/6 mice were used as the reference for comparison of sex- and species-dependent differences in P450 gene expression. In male mice, tissue-specific P450 expression patterns were similar between saline (Fig. 1A) and CO (Fig. 1C) vehicle controls. The liver expressed CYP2B10, 3A11, 1A2, and 2S1, but not CYP4X1, which is consistent with previous reports of brain-specific CYP4X1 expression in

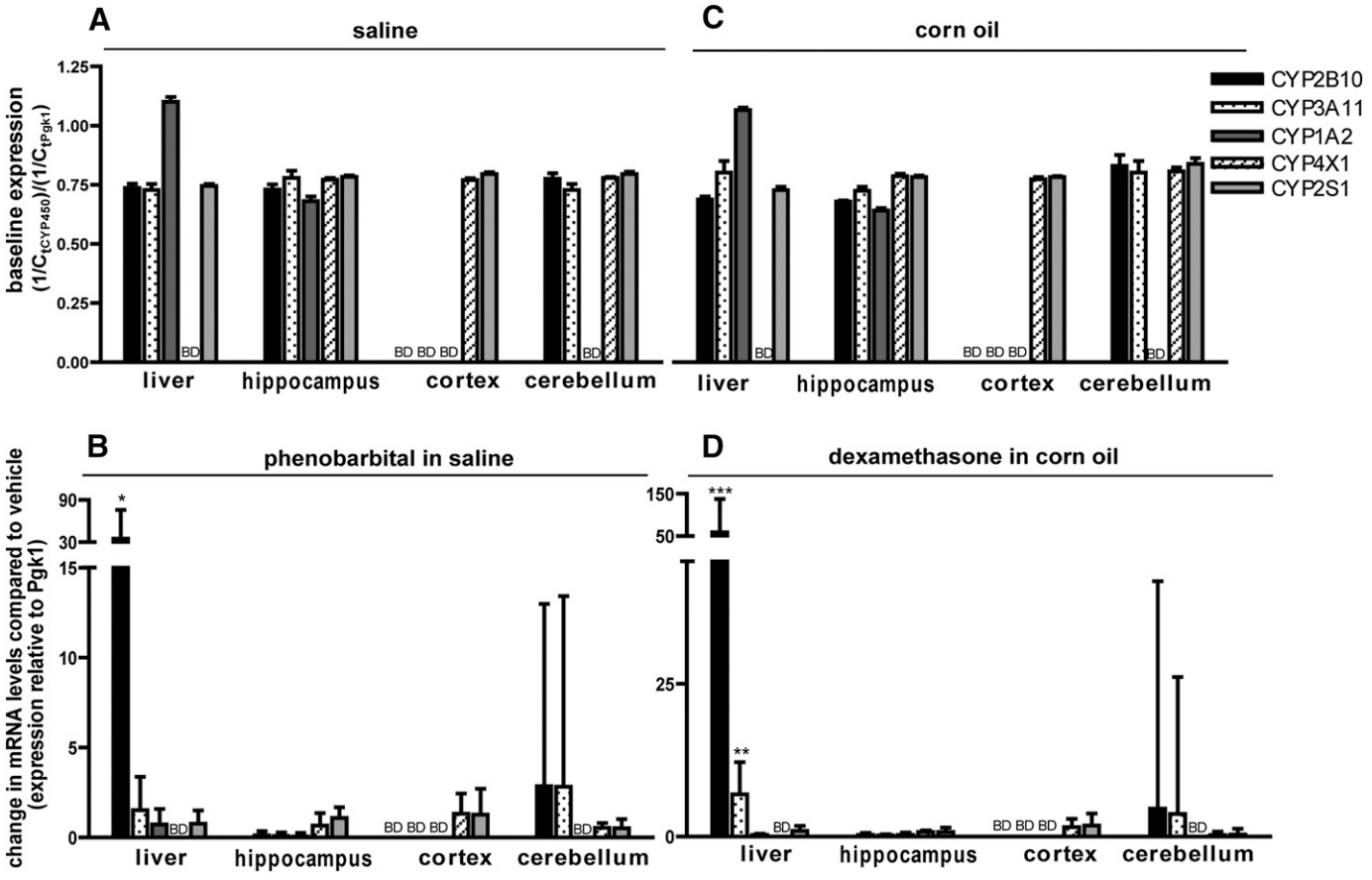

Fig. 1. P450 expression profiles in the brains of adult male C57BL/6 mice. Mice were treated for 3 consecutive days with either saline (20 ml/kg/day, i.p.) (A) or an equal volume of PB in saline (102 mg/kg/day, i.p.) (B), or for 4 consecutive days with either CO (10 ml/kg/day, i.p.) (C) or an equal volume of DEX in CO (50 mg/kg/day, i.p.) (D). Tissues were harvested 24 hours after the last injection and P450 mRNA quantified by quantitative (real-time) polymerase chain reaction. (A and C) Baseline P450 expression determined by normalizing fractional amplification (cycle number at which fluorescence exceeds a user-defined threshold) (Ct) values for P450 transcripts in control samples to Ct values for the reference gene [phosphoglycerate kinase 1 (Pgk1)] in the same sample. (B and D) Change in expression of the target gene in PB- or DEX-treated animals relative to vehicle control (saline for PB and CO for DEX). All data are expressed as the mean relative expression ± S.E. ($n = 4$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, significantly different from vehicle control as determined by automated randomization and bootstrapping tests (REST 2009 software). BD, below detection limit.

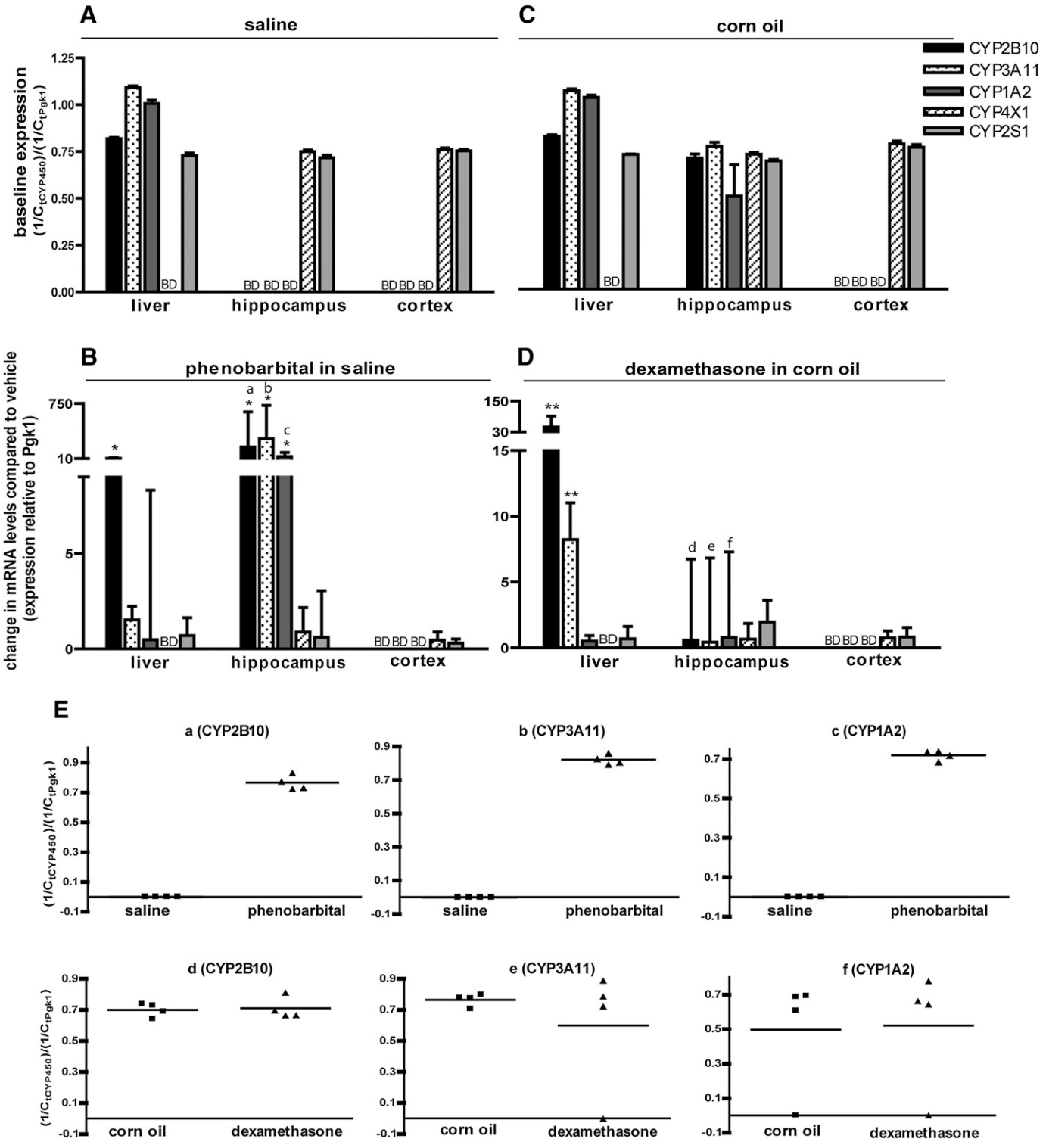

Fig. 2. P450 expression profiles in the brains of adult female C57BL/6 mice. Mice were treated for 3 consecutive days with either saline (20 ml/kg/day, i.p.) (A) or an equal volume of PB in saline (102 mg/kg/dat, i.p.) (B), or for 4 consecutive days with either CO (10 ml/kg/day, i.p.) (C) or an equal volume of DEX in CO (50 mg/kg/day, i.p.) (D). Tissues were harvested 24 hours after the last injection and P450 mRNA measured by quantitative (real-time) polymerase chain reaction. (A and C) Baseline P450 expression determined by normalizing fractional amplification (Ct) values for P450 transcripts in control samples to Ct values for phosphoglycerate kinase 1 (Pgk1) in the same sample. (B and D) Change in expression of the target gene in PB- or DEX-treated animals relative to vehicle control (saline for PB and CO for DEX). (E) Ct values for hippocampal CYP2B10, CYP3A11, and CYP1A2 in the PB group (a,b,c) and the DEX group (d,e,f) of individual mice. Data in (A–D) are expressed as the mean ± S.E. (n = 4–5). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, significantly different from vehicle controls as determined using the REST 2009 software. BD, below detection limit.

mice (Al-Anizy et al., 2006; Renaud et al., 2011). P450 expression profiles in the brain were region-specific: all five P450 isoforms were expressed in the hippocampus; the cerebellum expressed transcripts for all but CYP1A2, and the cortex expressed only CYP2S1 and CYP4X1 mRNA (Fig. 1, A and C). Treatment with either PB or DEX induced P450 expression in the liver (Fig. 1, B and D; see also Supplemental

Tables 4 and 5): hepatic CYP2B10 was induced by PB [mean of 35.1; 68% confidence interval (CI): 14.8–75.7] and by DEX (mean of 58.8; 68% CI: 30.6–137.5). DEX also induced hepatic CYP3A11 (mean of 6.9; 68% CI: 3.8–12.2). However, neither PB nor DEX induced expression of any target P450 in the hippocampus, cerebellum, or cortex (Fig. 1, B and D).

To explore the influence of sex, we measured P450 transcript levels in female C57BL/6 mice. Baseline P450 expression in the liver and cortex was similar between the saline (Fig. 2A) and CO (Fig. 2C) vehicle controls, and comparable to profiles observed in male vehicle controls (Fig. 1, A and C). Similar to males, all five P450 isoforms were expressed in the hippocampus of CO-treated females (Fig. 2C); however, in contrast to males, only CYP4X1 and CYP2S1 were detected in the hippocampus of saline-treated females (Fig. 2A). CO has been previously reported to influence P450 expression in the rat liver (Yoo et al., 1990), but it is unclear whether our findings reflect CO-mediated increase in P450 levels in the female mouse hippocampus. Although differences between saline- and CO-treated female mice could be experimental artifact, this seems unlikely because hippocampal expression levels of CYP4X1 and CYP2S1 were comparable between the two vehicle treatments and between sexes.

As observed in male mice, PB induced hepatic CYP2B10 expression in female mice by a mean factor of 13.2 (68% CI: 6.6–23.7), whereas DEX induced hepatic expression of CYP2B10 and CYP3A11 by 48.6 (68% CI: 30.6–91.1) and 8.21 (68% CI: 5.6–11.0), respectively (Fig. 2, B and D). Also consistent with male mice, PB or DEX did not change P450 expression in the cortex of female mice. Similarly, DEX had no effect on P450 expression in the female hippocampus. However, in contrast to males, PB significantly induced expression of CYP2B10 (mean of 164; 68% CI: 56–529), CYP3A11 (mean of 279; 68% CI: 113–724), and CYP1A2 (mean of 36; 68% CI: 14–94) in the hippocampus of females relative to saline vehicle controls (Fig. 2B). Data for these three transcripts are shown for individual female mice in Fig. 2E.

To investigate species-dependent differences, P450 transcripts were quantified in male rats. Tissue-specific P450 expression profiles were similar between saline (Fig. 3A) and CO (Fig. 3C) vehicle controls, but varied from those observed in the comparable male mouse treatment groups (Fig. 1, A and C). Specifically, in the male rat, all five P450 isoforms of interest were expressed in the liver, including CYP4X1 (Fig. 3, A and C). Also in contrast to male mice, rat brain expressed CYP3A2, CYP4X1, and CYP2S1 in the hippocampus, cortex, and cerebellum, but neither CYP2B1/2 nor CYP1A2 was detected in any of these three brain regions (Fig. 3, A and C). These findings are consistent with previous studies of regional P450 expression in rat brain, with the exception that others have reported the presence of

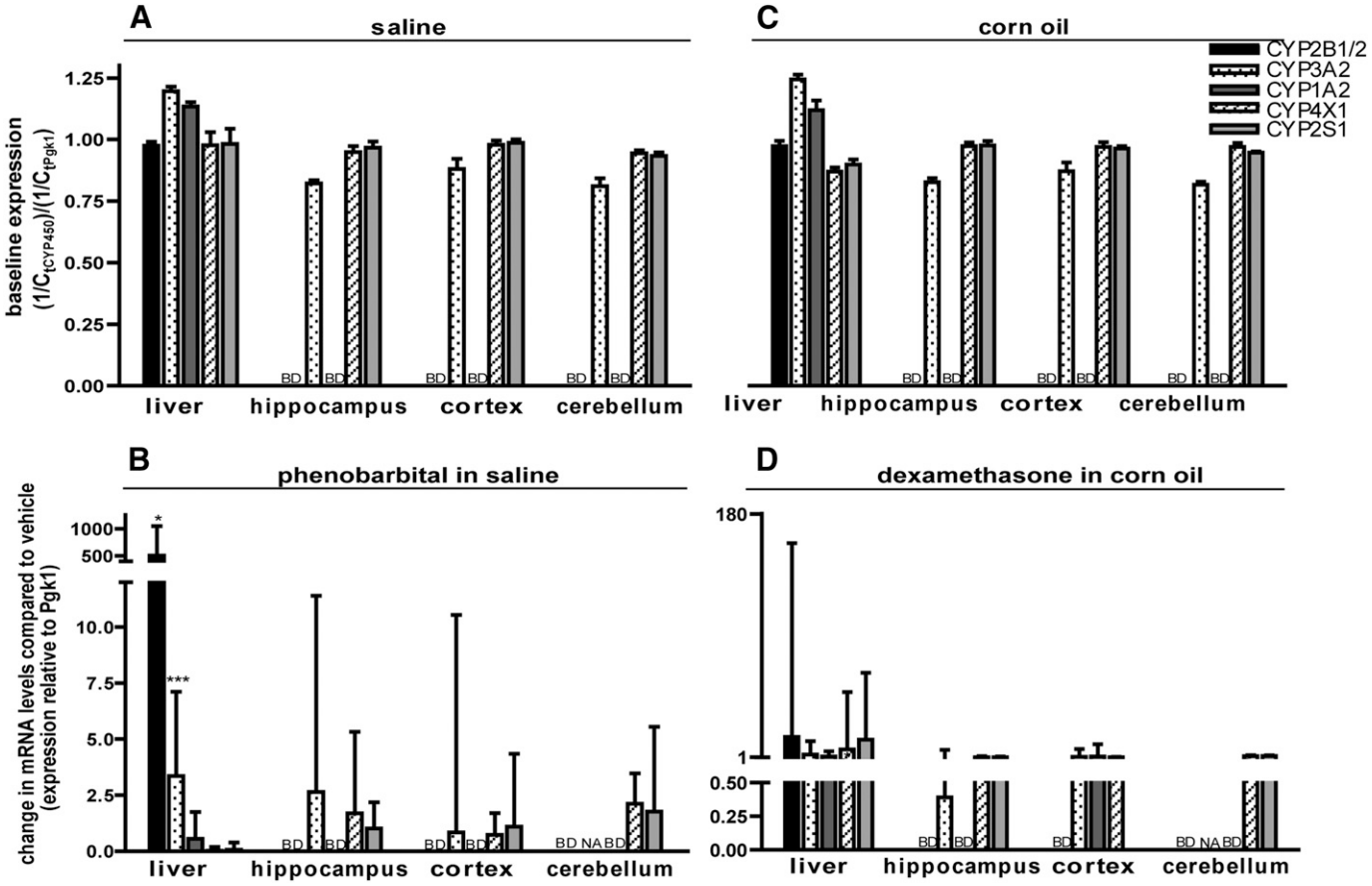

Fig. 3. P450 expression profiles in the brain of adult male Sprague-Dawley rats. Rats were treated for 3 consecutive days with either saline (5 ml/kg/day, i.p.) (A) or an equal volume of PB in saline (102 mg/kg/day, i.p.) (B), or for 4 consecutive days with either CO (5 ml/kg/day, i.p.) (C) or an equal volume of DEX in CO (50 mg/kg/day, i.p.) (D). Tissues were harvested 24 hours after the last injection and P450 mRNA quantified by quantitative (real-time) polymerase chain reaction. (A and C) Baseline P450 expression determined by normalizing fractional amplification (Ct) values for CYP transcripts in vehicle control tissues to Ct values for phosphoglycerate kinase 1 (Pgk1) in the same sample. (B and D) Change in expression of the target gene in PB- or DEX-treated animals relative to control (saline for PB and CO for DEX). Data are expressed as the mean ± S.E. ($n = 3$ except for PB-treated cerebellum, in which $n = 2$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, significantly different from vehicle controls as determined by automated randomization and bootstrapping tests (REST 2009 software). BD, below detection limit; NA, not available because of low amplification efficiency.

CYP2B in rat brain (Schilter and Omiecinski, 1993). Similar to male mice, PB (Fig. 3B) and DEX (Fig. 3D) induced P450 expression in the male rat liver but not in any of the three brain regions. However, the profile of hepatic P450 isoforms induced by these chemical treatments showed species variation. In the rat, PB induced the expression of not only CYP2B1/2 (by a mean factor of 504; 68% CI: 298–1044) but also CYP3A2 (by a mean factor of 3.4; 68% CI: 1.8–7.1). Surprisingly, DEX did not significantly alter hepatic CYP2B1/2 or CYP3A2 expression, but instead significantly upregulated CYP4X1 expression (by a mean factor of 6.5; 68% CI: 1.5–78.8). Although the latter is a novel finding, given the low fold-induction and the lack of protein expression data, the functional significance of this upregulation is not clear.

To further investigate sex- and species-specific differences in hepatic P450 induction, we compared relative CYP2B and CYP3A induction in the liver of male versus female mice and between male mice and male rats. We found no significant sex differences in P450 induction patterns in mice (Fig. 4A). Conversely, there were significant differences in hepatic P450 induction between mice and rats (Fig. 4B). Hepatic CYP2B expression was induced by DEX in mice but not rats, and CYP3A expression was induced by PB in mice but not rats and by DEX in rats but not mice.

In summary, these data suggest that P450 mRNA expression in the brain 1) differs significantly from hepatic P450 transcript profiles in rodent models; 2) varies between brain regions; 3) exhibits subtle sex-dependent differences in the C57BL/6 mouse, but significant species-specific differences between mouse and rat; and 4) with the possible exception of P450s in the hippocampus of the female mouse, is not induced by PB or DEX regimens that induce hepatic orthologs. With respect to the last finding, previous studies of whole brain homogenates have reported either no P450 induction by these classic inducers (Schilter et al., 2000; Hedlund et al., 2001; Upadhya et al., 2002; Woodland et al., 2008) or CYP2B induction by PB (Schilter and Omiecinski, 1993; Schilter et al., 2000; Upadhya et al., 2002). Discrepancies between studies likely reflect differences in dose and duration of treatment, species and/or strain, whole brain versus isolated brain regions, primer specificity, and methods of mRNA quantification. Although it will be necessary to confirm protein levels and activity of these P450 isoforms to corroborate the significance of these findings, emerging evidence of brain P450-mediated xenobiotic activation strongly suggests that differences in regional expression of brain P450s may be an important mechanism contributing to region-selective neurotoxicity (Spencer and Lein, 2013).

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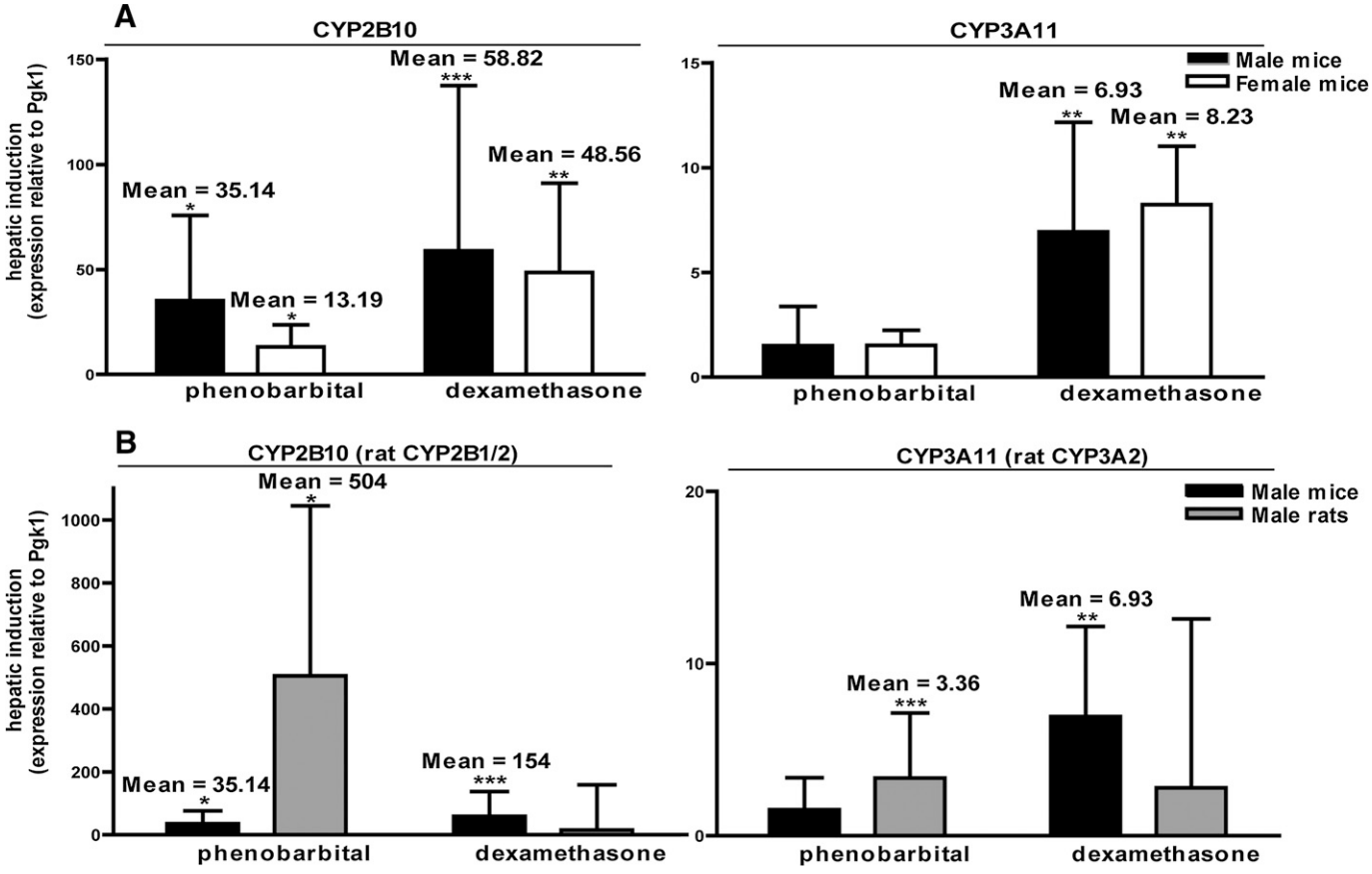

Fig. 4. P450 induction in liver is species-dependent but not sex-dependent. (A) In both male and female mice, hepatic CYP2B10 is significantly induced by both PB and DEX, whereas hepatic CYP3A11 is upregulated by DEX but not PB. Differences in induction between sexes are not statistically significant as determined by Student's *t* test ($P < 0.05$). (B) Hepatic CYP2B10 is induced by both PB and DEX in male mice, but the rat ortholog, CYP2B1/2, is induced only by PB in male rats. CYP3A11 is upregulated in male mice by DEX but not by PB. Conversely, the rat ortholog, CYP3A2, is induced by PB but not by DEX. P450 transcript levels in animals treated with either PB or DEX are presented relative to species-specific vehicle controls. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, significantly different from vehicle controls as determined by automated randomization and bootstrapping tests (REST 2009 software).

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Conducted experiments: Stamou, Wu, Kania-Korwel.

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Wrote or contributed to the writing of the manuscript: Stamou, Wu, Kania-Korwel, Lehmler, Lein.

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Detailed Materials and Methods

Total RNA extraction and reverse transcription

Following the manufacturer's instructions, total RNA was extracted from liver, hippocampus and cortex samples using RNeasy (Qiagen, Valencia, CA), and from cerebellum samples using Trizol (Invitrogen, Carlsbad, CA). Following digestion of total RNA with 100 U of DNase I (Invitrogen), 1 µg of total RNA per sample was mixed with 200 U of Superscript III reverse transcriptase and 50 ng/µl of random hexamer primers and reverse transcribed to cDNA using the Superscript III First Strand Synthesis kit (Invitrogen) according to the manufacturer's protocol. The OD_{260nm}/OD_{280nm} of resultant cDNA was confirmed to be >1.8.

Quantitative polymerase chain reaction (qPCR)

Primer and probe sets for specific CYP isoforms (Table S6) were designed in-house using PrimerBlast from NCBI (Bethesda, MD) and PrimerQuest software (IDT, Coralville, IA). Separate primer and probe sets were designed for mouse *versus* rat CYP genes. Specificity of the primers and probes for each gene was confirmed by BLASTN searches conducted against nucleotide collection databases for *Mus musculus* and *Rattus norvegicus*. CYP isoform-specific primer and probe sets were synthesized by IDT, which was also the source of a commercially available primer and probe set for the reference gene phosphoglycerate kinase 1 (Pgk1). Pgk1 has previously been reported to be a stable reference gene for qPCR analyses of brain samples (Santos and Duarte, 2008), and we similarly found stable Pgk1 expression across all species, tissues and tissue regions, irrespective of treatment. All fluorescent probes contained a ZEN internal quencher (IDT) to eliminate background fluorescence.

qPCR was performed using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using the Taqman Universal PCR Master Mix (Life Sciences, Grand Island, NY) according to the manufacturer's instructions. Thermal cycling conditions consisted of an initial annealing step at 50°C for 2 min, followed by a denaturation step at 95°C for 10 min and 40 cycles at 95°C for 15 sec and 60°C for 1 min. After synthesis, PCR products were subjected to 1% agarose gel electrophoresis to confirm that products were of the expected band size. No-template and no-enzyme controls were run with each assay and confirmed to produce negligible signal (> 39 Ct value where Ct is defined as the fractional amplification cycle number at which fluorescence exceeds a defined threshold). Samples from saline vehicle controls were used to construct dilution curves for each CYP transcript and estimate amplification efficiencies for each tissue from male and female mice and male rats. The amplification efficiency for each gene was calculated using the Pfaffl equation (Pfaffl, 2001): $E = 10^{-1/slope} - 1$.

Analysis of qPCR data

Analysis of amplification results was performed using the 7500 Fast System SDS software (Applied Biosystems) to obtain Ct values (Pfaffl et al., 2002). For all samples in the vehicle control groups (saline and corn oil treatments), the Ct value obtained for each CYP transcript was normalized to the Ct value for the reference gene (Pgk1) within the same sample. Since high Ct values correspond to low transcript levels, reciprocal Ct values for each gene were normalized to the reciprocal Ct values for Pgk1 in samples from vehicle controls to assess baseline CYP transcript expression across tissues. Two-way *ANOVA* with *post hoc* Bonferroni test was used identify significant vehicle effects on transcript expression levels.

CYP expression levels in animals treated with phenobarbital (PB) or dexamethasone (DEX) were compared to those of animals treated with the appropriate vehicle, which was saline for PB and corn oil for DEX. Relative differences in gene expression between experimental

groups are often determined using the delta-delta-Ct (ddCt) algorithm (Livak and Schmittgen, 2001). However, this algorithm assumes that the amplification efficiencies of the target gene and the reference gene are identical. To assess whether this condition was met in our study, we first constructed dilution curves and calculated the amplification efficiency for each gene in each tissue of interest harvested from saline-treated animals. As shown in Table S1, amplification efficiencies were different between the CYP genes and our reference gene; therefore, we could not use the ddCt algorithm. Instead, expression levels in PB- or DEX-treated animals relative to the appropriate vehicle control animals (saline for PB and CO for DEX) were analyzed using the REST2009 software (Qiagen), which employs an amplification efficiency corrected calculation model based on multiple samples according to the following formula (Pfaffl, 2001; Pfaffl et al., 2002):

Relative expression = (concentration of CYP target gene) / (concentration of Pgk1)

In this formula, concentration = efficiency$^{\Delta Ct}$. The ΔCt value for each gene was determined by subtracting the average Ct value of the gene of interest in the control sample from the average Ct value of the same gene in the treated sample. The REST2009 software determines the statistical significance of calculated expression ratios using randomization algorithms (random pairing of controls and samples from the gene of interest and the reference gene, and calculation of their expression ratio).

References

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:**402-408.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29:**e45.

Pfaffl MW, Horgan GW, and Dempfle L (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* **30:**e36.

Santos AR and Duarte CB (2008) Validation of internal control genes for expression studies: effects of the neurotrophin BDNF on hippocampal neurons. *Journal of neuroscience research* **86:**3684-3692.

Table S1: Treatment effects on body and liver weights of male mice[a]

Treatment	Final weight [g]	Body weight change	Liver weight [g]	Liver-to-body weight ratio
Phenobarbital (n=4)	21.9 ± 1.7	2.1 ± 3.4	1.4 ± 0.1	6.2 ± 0.5*
Saline (n=5)	22.9 ± 2.3	0.1 ± 0.4	1.1 ± 0.1	4.8 ± 0.3
Dexamethasone (n=4)	23.1 ± 1.5	-0.6 ± 0.5	1.9 ± 0.4*#	8.3 ± 1.1*#
Corn oil (n=5)	24.3 ± 0.9	-0.2 ± 0.3	1.2 ± 0.1	4.8 ± 0.1

[a]Values represent the means ± standard deviation. *Significantly different from vehicle control (saline for phenobarbital; corn oil for dexamethasone) at $p < 0.5$; #significantly different from phenobarbital at $p < 0.5$ (paired Student's t-test, SAS version 9.3).

Table S2: Treatment effects on body and liver weights of the female mice[a]

Treatment	Final weight [g]	Body weight change	Liver weight [g]	Liver-to-body weight ratio
Phenobarbital (n=4)	18.3 ± 1.3	-1.8 ± 1.0	1.8 ± 0.2*	9.9 ± 1.1*
Saline solution (n=5)	17.0 ± 1.1	-0.7 ± 0.4	1.3 ± 0.2	7.4 ± 1.2
Dexamethasone (n=5)	18.8 ± 0.9	0.7 ± 0.7#	1.6 ± 0.3*	8.7 ± 1.2*
Corn oil (n=4)	18.0 ± 0.4	-0.1 ± 0.1	1.1 ± 0.1	6.0 ± 0.5

[a]Values represent means ± standard deviation. *Significantly different from vehicle control at $p < 0.5$ (saline for phenobarbital; corn oil for dexamethasone); #significantly different from phenobarbital at $p < 0.5$ (paired Student's t-test, SAS version 9.3).

Table S3: Treatment effects on body and liver weights of male rats[a]

Treatment	Final weight [g]	Body weight change	Liver weight [g]	Liver-to-body weight ratio
Phenobarbital (n=3)	314.0 ± 11.4	7.3 ± 7.6	15.4 ± 1.5	4.9 ± 0.3*
Saline (n=5)	306.6 ± 5.9	3.2 ± 3.5	12.8 ± 0.5	4.2 ± 0.1
Dexamethasone (n=5)	238.0 ± 11.0*#	-57.0 ± 4.0*#	16.0 ± 1.3*#	6.7 ± 0.3*#
Corn oil (n=5)	298.8 ± 6.2	1.8 ± 1.9	12.7 ± 1.0	4.3 ± 0.3

[a]Values represent means ± standard deviation. *Significantly different from vehicle control at $p < 0.5$ (saline for phenobarbital; corn oil for dexamethasone); #significantly different from phenobarbital at $p < 0.5$ (paired Student's t-test, SAS version 9.3).

Table S4: Fold-change in hepatic cytochrome P450 enzyme mRNA in phenobarbital-induced animals relative to saline vehicle controls

	Male C57Bl/6 mice			Female C57Bl/6mice			Male Sprague Dawley rats		
CYP enzyme	Relative expression	Standard error	95% confidence interval	Relative expression	Standard error	95% confidence interval	Relative expression	Standard error	95% confidence interval
Murine CYP2B10 (rat CYP2B1/2)	35.1*	14.8-75.7	9.6-134	13.2*	6.6-23.7	4.5-31.0	504*	298-1044	156-1290
Murine CYP3A11 (rat CYP3A2)	1.5	0.7-3.4	0.4-5.1	1.5	1.0-2.2	0.8-2.9	3.4	1.8-7.1	1.2-11.3
CYP1A2	0.7	0.3-1.6	0.2-3.4	0.5	0.0-8.3	0.0-18.5	0.6	0.3-1.8	0.1-2.4
CYP4X1							0.0	0.0-0.2	0.0-0.4
CYP2S1	0.8	0.5-1.5	–0.2-2.0	0.7	0.2-1.6	0.1-5.9	0.1	0.0-0.4	0.0-0.8

*Indicates statistically significant change (upregulation) of gene expression in phenobarbital-induced animals compared to expression in saline vehicle animals ($p<0.05$). The relative expression is calculated as the ratio of the concentration of the target gene compared to the concentration of the reference gene (Pgk1). Concentrations, relative expression, standard error and 95% confidence intervals were calculated using the REST2009 software (Qiagen, Valencia, CA), which incorporates Ct and efficiency values determined by qPCR analysis.

Table S5: Fold-change in hepatic cytochrome P450 enzyme mRNA in dexamethasone-induced animals relative to corn oil vehicle controls

	Male C57Bl/6 mice			Female C57Bl/6mice			Male Sprague Dawley rats		
CYP enzyme	Relative expression	SE	95% confidence interval	Relative expression	SE	95% confidence interval	Relative expression	SE	95% confidence interval
Murine CYP2B10 (rat CYP2B1/2)	58.8*	30.6-137	15.5-205	48.6*	30.6-91.0	18.8-115	15.7	1.7-158	0.7-302
Murine CYP3A11 (rat CYP3A2)	6.9*	3.8-12.2	2.5-18.8	8.2	5.6-11.0	4.8-15.3	2.8	0.8-12.6	0.5-24.5
CYP1A2	0.3	0.2-0.5	0.1-0.6	0.5	0.2-0.9	0.2-1.7	1.2	0.3-5.2	0.1-14.2
CYP4X1							6.5*	1.5-48.8	1.2-95.6
CYP2S1	1.0	0.4-1.8	0.3-2.0	0.7	0.3-1.6	0.2-2.1	13.2	3.0-60.4	2.0-195

*Indicates statistically significant change (upregulation) of gene expression in dexamethasone-induced animals compared to expression in corn oil-treated animals ($p<0.05$). The relative expression is calculated as the ratio of the concentration of the target gene compared to the concentration of the reference gene (Pgk1). Concentrations, relative expression, standard error and 95% confidence intervals were calculated using the REST2009 software (Qiagen, Valencia, CA), which incorporates Ct and amplification efficiency values determined by qPCR analysis.

Table S6. CYP-specific primer set sequences

Human CYP isoform	**Mouse orthologue**		**Rat orthologue**	
CYP2B6	**CYP2B10**		**CYP2B1/2**	
	Forward primer	5'CCAAATCTCCAGGGCTCCAAGGC3'	Forward primer	5' CAACCCTTGATGACCGCAGT3'
	Reverse primer	5'TGCGGACTTGGGCTATTGGGAGG3'	Reverse primer	5' TGGAGAGCTGAACTCAGGATGGG3'
CYP3A4	**CYP3A11**		**CYP3A2**	
	Forward primer	5'ACAAGCAGGGATGGACCTGGTT3'	Forward primer	5' AATGGAGCCTGACTTTCCCTCAAG3
	Reverse primer	5'CCCATATCGGTAGAGGAGCACCA3'	Reverse primer	5'GCATCAAGAGCAGTCAATTAAGTCCCAG3'
CYP1A2	**CYP1A2**		**CYP1A2**	
	Forward primer	5'CCAGCCCCTGCCCTTCAGTGGTA3'	Forward primer	5' ATGAAGCCCAGAACCTGTGAAC3'
	Reverse primer	5'TGGGAACCTGGGTCCTTGAGGC3'	Reverse primer	5' GTATGGGTTTGCAGGGAACAGT3'
CYP4X1	**CYP4X1**		**CYP4X1**	
	Forward primer	5'CACCCTTGTGCCTTCCCCTGC3'	Forward primer	5' AAACGGCACCTATGAGTCTTATG3'
	Reverse primer	5'CCTCGTCCAATGCATGGAGTCAGG3'	Reverse primer	5' TTGCCTAACTCCTGGAAGCA3'
CYP2S1	**CYP2S1**		**CYP2S1**	
	Forward primer	5'TCGGGGCTTTTGCGGCTAAGT3'	Forward primer	5' AGGACGTCCATTCAACCCTTCCAT3'
	Reverse primer	5'CAACCAGGACCACCACGCGG3'	Reverse primer	5' TCATAGGGCAAACGGATGCCAAAG 3'

Table S7. Amplification efficiency[a] of Pgk1 (reference gene) and CYP genes

	Pgk1		**CYP2B10**		**CYP3A11**		**CYP1A2**		**CYP4X1**		**CYP2S1**	
	R^2	E[b]	R^2	E	R^2	E	R^2	E	R^2	E	R^2	E
Male C57BL/6 mice												
Liver	0.997	110%	0.997	64%	0.991	80%	0.974	89%	ND[c]		0.953	113%
Hippocampus	0.986	109%	0.96	104%	0.952	80%	0.940	98%	0.983	90%	0.988	113%
Cortex	0.975	112%	ND		ND		ND		0.952	59%	0.987	102%
Cerebellum	0.932	113%	0.975	96%	0.982	67%	ND		0.983	93%	0.991	104%
Female C57BL/6 mice[d]												
Liver	0.988	115%	0.982	100%	0.987	84%	0.995	102%	ND		0.985	115%
Hippocampus	0.998	95%	ND[e]		ND[e]		ND[e]		0.970	82%	0.976	82%
Cortex	0.997	77%	ND		ND		ND		0.993	77%	0.994	81%
Male Sprague Dawley rats												
Liver	0.954	89%	0.992	96.50%	0.998	96.60%	0.995	94%	0.985	96%	0.983	103%
Hippocampus	0.969	89%	ND		0.96	87%	ND		0.987	90%	0.95	105%
Cortex	0.941	104%	ND		0.959	93.60%	0.991	95%	0.987	102%	0.997	102%
Cerebellum	0.972	111%	ND		ND		ND		0.971	94%	0.975	98%

[a]Amplification efficiency was determined from tissues of saline-treated animals; [b]E = efficiency; [c]ND = not detected; [d]samples from cerebellum of female mice were not used due to the low quality of total RNA extracted from these tissues; [e]in the absence of expression in the saline-treated animals, upregulation of this CYP was calculated using the amplification efficiency for the same CYP from male mice.