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^oC for 2 min, followed by a denaturation step at 95^oC for 10 min and 40 cycles at 95^oC for 15 sec and 60^oC 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.