

ORIGINAL ARTICLE

Decreased hippocampal volume and increased anxiety in a transgenic mouse model expressing the human *CYP2C19* geneA Persson¹, SC Sim¹, S Viriding¹, N Onishchenko², G Schulte³ and M Ingelman-Sundberg¹

Selective serotonin reuptake inhibitors, tricyclic antidepressants, various psychoactive drugs, as well as endogenous steroids and cannabinoid-like compounds are metabolized by the polymorphic cytochrome P450 2C19 (*CYP2C19*). Absence of this enzyme has been recently shown to associate with lower levels of depressive symptoms in human subjects. To investigate endogenous functions of *CYP2C19* and its potential role in brain function, we have used a transgenic mouse model carrying the human *CYP2C19* gene. Here, *CYP2C19* was expressed in the developing fetal, but not adult brain and was associated with altered fetal brain morphology, where mice homozygous for the *CYP2C19* transgenic insert had severely underdeveloped hippocampus and complete callosal agenesis and high neonatal lethality. *CYP2C19* expression was also found in human fetal brain. In adult hemizygous mice we observed besides decreased hippocampal volume, an altered neuronal composition in the hippocampal dentate gyrus. Reduced hippocampal volumes have been reported in several psychiatric disorders, supporting the relevance of this model. Here we found that adult hemizygous *CYP2C19* transgenic mice demonstrate behavior indicative of increased stress and anxiety based on four different tests. We hypothesize that expression of the *CYP2C19* enzyme prenatally may affect brain development by metabolizing endogenous compounds influencing this development. Furthermore, *CYP2C19* polymorphism may have a role in interindividual susceptibility for psychiatric disorders.

Molecular Psychiatry (2014) **19**, 733–741; doi:10.1038/mp.2013.89; published online 23 July 2013

Keywords: antidepressants; anxiety; *CYP2C19*; depression; hippocampus; stress

INTRODUCTION

Hippocampal dysfunction is believed to have a crucial role in the pathophysiology of both major depressive disorder (MDD) and anxiety-related disorders.^{1,2} Reduced hippocampal volumes have been reported in several psychiatric disorders including anxiety-related post-traumatic stress disorder and schizophrenia.^{2–4} However, the phenotype has been most extensively studied in MDD patients,^{5,6} where hippocampal volume reduction has been shown to correlate with the severeness and duration of the disorder.^{7,8}

In a cohort of 1500 European subjects, we recently found using the Center for Epidemiologic Studies Depression Scale a genetic association of individuals lacking cytochrome P450 2C19 (*CYP2C19*) enzyme function with lower levels of depressive symptoms.⁹ *CYP2C19* is involved in the metabolism of several psychotropic drugs including the selective serotonin reuptake inhibitors sertraline and citalopram, the tricyclic antidepressants amitriptyline and clomipramine,¹⁰ as well as benzodiazepines.¹¹ This indicates that *CYP2C19* is able to metabolize substrates with a CNS drug-effect profile. Endogenous compounds suggested as substrates for *CYP2C19* based on *in vitro* studies include psychoactive steroid hormones such as estradiol,¹² estrone,¹³ progesterone and testosterone.¹⁴

The *CYP2C19* gene is polymorphic and the *CYP2C19*17* allele, present at 18–27% in different European populations,^{15–17} causes higher gene expression and more rapid drug metabolism,¹⁵ whereas *CYP2C19*2* with an allele frequency of around 15% in Caucasians¹⁸ is the most common null allele.¹⁰ In Asian

populations, the allele frequency of the *CYP2C19*17* allele is approximately 6% and the *CYP2C19*2* allelic variant has a frequency of about 30%.¹⁸ *CYP2C19* polymorphism impacts not only drug plasma levels,^{19–21} but also the therapeutic outcome of psychotropic drugs.²²

In light of the association of *CYP2C19* polymorphism with depressive symptoms,⁹ we investigated brain-related effects of transgenic expression of the human *CYP2C19* gene in mice. We found that the *CYP2C19* gene is expressed in mouse and human fetal brain. The transgenic animals exhibited altered hippocampal morphology both at young and adult age, as well as changes in hippocampal neuronal composition in adult life. In line with these findings, we observed behavioral changes related to stress and anxiety in adult mice. Our data suggest a role for *CYP2C19* in brain development, and the human *CYP2C19* transgenic mouse model might provide a useful tool for studies of hippocampal function and stress and anxiety-related phenotypes.

MATERIALS AND METHODS

For detailed methodological descriptions, see Supplementary Information 1.

Transgenic mice

A C57BL/6 mouse line transgenic for a BAC insert containing the human *CYP2C18* and *CYP2C19* genes has previously been produced and estimated to hemizygously (*CYP2C19Tg-Hem*) carry 12 copies of the insert at region C1 of mouse chromosome 2.^{23,24} All experiments were carried out on male mice, with the exception of unknown sex status of mice during fetal

¹Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; ²Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden and ³Section of Receptor Biology and Signaling, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. Correspondence: Dr M Ingelman-Sundberg, Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, SE 171 77, Sweden. E-mail: magnus.ingelman-sundberg@ki.se

development. All experiments were approved by the local ethical committee; Stockholm Northern Ethics Board of Animal Experimentation.

CYP2C18 and *CYP2C19* expression in transgenic mice and human fetal brain

In *CYP2C19* transgenic mice, *CYP2C18* and *CYP2C19* mRNA expression was investigated in brain and liver tissue during embryonic development (embryonic day 14 (E14) and E18), early postnatal days (PND0 and PND7) and at 7 weeks of age, using real-time PCR protocols obtained from Löfgren et al.²⁴ Four to five mice were analyzed for each time point. Human brain tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, USA and were from three different female Caucasian donors; gestational week 19, 24 and 39.

Brain morphology

Mice (PND0, 7 and 15 weeks) were anesthetized and perfused transcardially with 4% paraformaldehyde solution, after which the brains were post-fixed, cryoprotected and snap-frozen. Coronal sections (40 μ m) were stained with cresyl violet for morphological assessment. For PND0 pups, three wild-type (Wt) brains were compared with four *CYP2C19Tg-Hom* brains. In 7- and 15-week old mice, 5–7 brains were analyzed per group.

Magnetic resonance imaging

In vivo magnetic resonance imaging (MRI) was conducted in anesthetized 15-week-old mice using a horizontal 9.4T Varian magnet ($n = 7$ per group). Hippocampal and whole-brain volumes (including cerebellum) were manually outlined using ITK-SNAP (www.itksnap.org).²⁵

BrdU injections and immunohistochemistry analyses of the hippocampus

Bromodeoxyuridine (BrdU) is widely used to evaluate hippocampal proliferation rates.²⁶ Seven-week-old mice were intraperitoneally injected with 75 mg kg⁻¹ BrdU; either twice, with a 16-h gap, and killed 2 h after the last injection, or injected once a day for 3 consecutive days and killed 4 weeks after the first injection. Brain sections were stained by immunohistochemistry (IHC) using Ki-67 or BrdU antibodies. Antibodies for parvalbumin and double-cortin (DCX) were used for IHC analyses of brain sections of 15-week-old mice not subjected to BrdU injections. Five to eight mice were analyzed per group. For all IHC markers, total numbers were also corrected for total hippocampal volumes as measured by MRI.

Behavioral tests

Behavioral tests were conducted in adolescent (7-week-old) and adult (15-week-old) mice. Depression-like behavior was evaluated in the tail-suspension test (TST; $n = 6$ –10 per group) and spatial learning was evaluated in 15-week-old mice using the Morris water maze (MWM) with a subsequent TST (Wt: $n = 10$; *CYP2C19Tg-Hem*: $n = 9$). Motor activity and anxiety levels were studied using the open-field and the light–dark box test ($n = 15$ per group). Stress response was studied by subjecting mice to 30 min of acute restraint, followed by direct decapitation or a 30-min recovery period before decapitation. Mice not subjected to stress were decapitated directly and served as controls. Serum corticosterone (CORT) was quantified and hippocampal *c-fos* mRNA expression was analyzed.

Statistical analysis

Statistical analyses were carried out using unpaired, two-tailed Student's *t*-test in GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Data are presented as mean \pm s.e.m. For correlation studies, Pearson's correlation test was performed. Statistical significance was defined as $P < 0.05$.

RESULTS

CYP2C19 expression in brain and liver tissue of transgenic mice and in human fetal brain

Human *CYP2C18* and *CYP2C19* mRNA and protein expression has previously been examined in adult transgenic (previously named *CYP2C19Tg*) mice (26–31 weeks). The highest mRNA expression

was found in liver for both genes, whereas the protein could only be detected for *CYP2C19* and not for *CYP2C18*,²⁴ similar to the relative protein expression of human liver.^{27,28} Examination of *CYP2C19* mRNA expression during development revealed relatively high levels in the brain at E14, E18 and PND0, with no significant hepatic expression at the same time points (Figure 1a). The mRNA expression in hippocampus, cortex and cerebrum were similar at day 18 (Supplementary Figure 1). At 7 weeks of age, the tissue-specific *CYP2C19* mRNA expression was silenced in brain and induced in liver (Figure 1b). Expression of the *CYP2C18* gene was found consistently low in the brain irrespective of the age (Figures 1a and b). Mice do not have *CYP2C19* or *CYP2C18* homologs, and thus, the observed human *CYP2C19* expression is specific for the transgenic mice. Quantification of *CYP2C19* mRNA in human fetal brain regions from three donors showed that *CYP2C19* can be expressed at a level representing slightly $> 1/200$ of human adult liver. Three fetal human brain donors, gestational weeks 19, 24 and 39, with a total of seven small cerebral brain pieces were analyzed for mRNA expression. The donor with the highest *CYP2C19* expression was in gestational week 24 and showed approximately 0.3% and 0.6% of the levels in adult human liver in two separate regions, respectively.

Altered brain morphology in *CYP2C19* transgenic mice

Homozygous mice (*CYP2C19Tg-Hom*) did not survive past PND3, and therefore brain morphology was investigated at PND0 in Wt, *CYP2C19Tg-Hem* and *CYP2C19Tg-Hom* pups. *CYP2C19Tg-Hom* pups were found to display distinct morphological abnormalities. The hippocampus in *CYP2C19Tg-Hom* pups was severely malformed (Figures 1e and f). Furthermore, connections between the hemispheres were severely underdeveloped in the *CYP2C19Tg-Hom* pups, with complete callosal agenesis (Figures 1c–e). The morphology of *CYP2C19Tg-Hem* brains were not different from Wt counterparts (data not shown).

Based on the abnormal brain morphology of newborn *CYP2C19Tg-Hom* mice, with a severely reduced hippocampal area (about 60%, Supplementary Figure 2), we performed volume measurements in adolescent and adult *CYP2C19Tg-Hem* male mice using both histology and MRI. In brain sections, a reduced hippocampal area was observed in *CYP2C19Tg-Hem* compared with Wt mice, both at 7 weeks (12% reduction; $P = 0.0079$) and 15 weeks of age (11% reduction; $P = 0.0082$; Figure 2a). Systematic sectioning revealed a prominent reduction in the caudal-ventral part of the hippocampus (Figures 2b and c). At both 7 and 15 weeks of age, a respective 5.4% ($P = 0.007$) and 5.6% ($P = 0.0004$) reduction of total brain weight was recorded in *CYP2C19Tg-Hem* compared with Wt mice (Supplementary Figure 3).

Fifteen-week-old *CYP2C19Tg-Hem* and Wt male mice were also examined using a 9.4 T Varian MRI system. Volumetric analyses showed decreased whole-brain (3.1%; $P = 0.018$) and hippocampal volumes (7.1%; $P = 0.013$) in *CYP2C19Tg-Hem* compared with Wt mice (Figures 3c and d). We found no correlation between whole-brain and hippocampal volumes ($r^2 = 0.276$; $P = 0.526$, Pearson's correlation test). Hippocampal volumes were therefore not normalized against whole-brain volumes.

Impact of human *CYP2C19* expression on mouse hippocampal cell populations

To investigate hippocampal plasticity in transgenic mice, IHC analyses assessing immature neurons were performed by detecting DCX-positive cells in 15-week-old male mice. We observed a marked decrease in this cell population, with a mean reduction as high as 42% ($P = 0.0002$) in *CYP2C19Tg-Hem* compared with Wt mice (Figures 4a, b and e). No difference in cell proliferation, as measured by BrdU and Ki-67-positive cells in the dentate gyrus (DG) of the hippocampus was observed in 7- or 15-week-old mice (Supplementary Figure 4).

Furthermore, we observed a reduction in the parvalbumin-positive γ -aminobutyric acid (GABA) interneuron population in the DG of 15-week-old *CYP2C19Tg-Hem* mice compared with Wt mice (22%, $P = 0.017$; Figures 4c, d and f) but no difference within the CA regions of the hippocampus (data not shown). Correction for mean hippocampal volume as measured by MRI (Supplementary Figure 5) revealed retained significance differences for all IHC markers with the only exception of parvalbumin-positive cells in the DG that showed border-line significance after size correction ($P = 0.071$; Supplementary Figure 5).

Behavioral phenotypes observed in transgenic mice

Using the TST, a classical test for assessing antidepressant drugs,²⁹ we found that 15-week-old *CYP2C19Tg-Hem* mice had a 55% lower immobility time compared with Wt controls ($P = 0.0017$; Figure 5c), whereas no differences were observed between groups of 7-week-old mice.

In the MWM, 15-week-old *CYP2C19Tg-Hem* mice acquired a despair-related behavior during the training week as evident by increased floating time compared with Wt mice (Supplementary Figure 6). Owing to this behavior, conclusive results on spatial

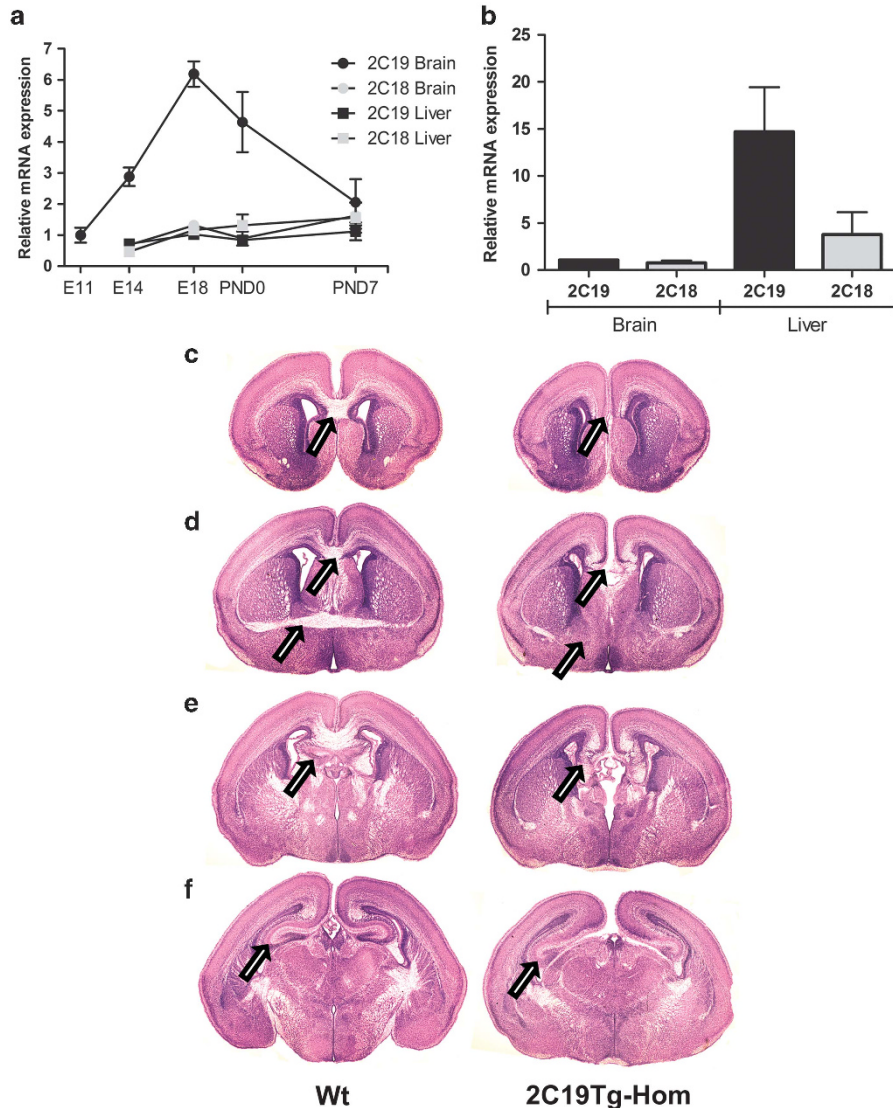


Figure 1. Human *CYP2C19* mRNA expression in developing *CYP2C19* transgenic (*CYP2C19Tg*) brain and the morphological brain phenotype at postnatal day 0 (PND0). **(a)** *CYP2C18* and *CYP2C19* mRNA expression pattern was investigated in brain and liver of hemizygous transgenic (*CYP2C19Tg-Hem*) mice during embryonic development (embryonic day 11 (E11), E14 and E18), early postnatal days (PND0 and PND7). The graph displays relative *CYP2C18* and relative *CYP2C19* mRNA expression levels in fetuses and pups of unknown gender. Brain *CYP2C19* mRNA expression peaks at E18, with a sixfold higher expression in brain compared with liver. The expression of the *CYP2C18* gene was consistently low in both tissues. **(b)** Relative *CYP2C18* and relative *CYP2C19* mRNA expression levels in 7 weeks old male mice. As evident from the graph, there is a brain-specific expression of *CYP2C19* mRNA in fetal life, which at the age of 7 weeks is silenced and hepatic expression induced. No expression of *CYP2C18* or *CYP2C19* mRNA was detected in wild-type (Wt) mice. Four to five mice were analyzed for each time point. Data are presented as mean with s.e.m. **(c–f)** Images are representative cresyl violet stainings of Wt ($n = 3$) and *CYP2C19Tg-Hom* ($n = 4$) pups brain sections at PND0. Brain sections from *CYP2C19Tg-Hem* mice were indistinguishable from those of Wt animals. The representative images display coronal sections from four rostral to caudal positions **(c–f)** per genotype. *CYP2C19Tg-Hom* mice display an extensive morphological phenotype with complete callosal agenesis **(c–e)**. As pointed out by arrows on each image, the corpus callosum **(c and top arrow d)**, the hippocampal commissure **(d, e)** and the anterior commissure (bottom arrow **d**), are all essentially lacking axons crossing over the midline of the hemispheres. Furthermore, the development of the hippocampal formation appears stalled or delayed in *CYP2C19Tg-Hom* mice **(e, f)**, with the different layers of the hippocampus being less distinct, in addition to an overall smaller appearance.

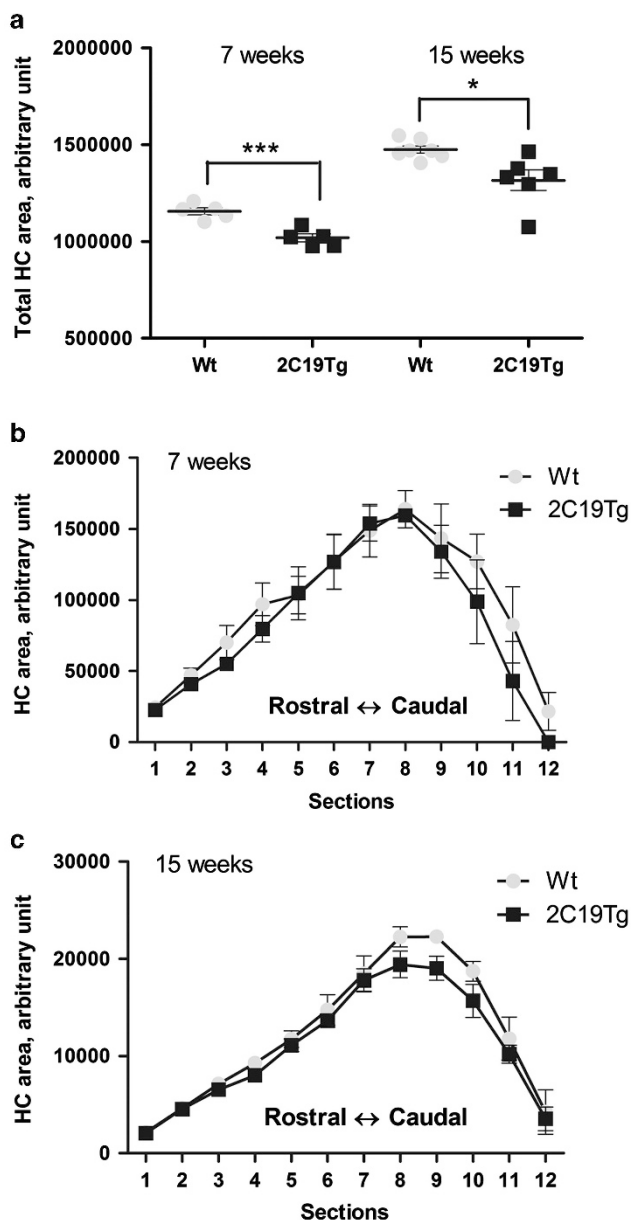


Figure 2. Decreased hippocampal area in adolescent and adult CYP2C19Tg-Hem compared with wild-type (Wt) mice. To investigate hippocampal morphology in young (7 weeks) and adult mice (15 weeks), 12 coronal brain sections from every mouse were stained with cresyl violet and the hippocampal area outlined and measured for each section. **(a)** A reduced total hippocampal area was observed in CYP2C19Tg-Hem compared with Wt male mice, both at 7 weeks (11.9% reduction; $P = 0.008$) and at 15 weeks of age (10.7% reduction; $P = 0.008$). **(b, c)** In the same mice as above **(a)** the hippocampal area is visualized per section in a rostral to caudal manner in 7- and 15-week-old mice, which highlights the specific reduction in size in the caudal part of the hippocampus at both ages. Data are presented as mean with s.e.m. * $P < 0.05$; *** $P < 0.001$. HC, hippocampal.

learning could not be obtained from the MWM test. However, when excluding transgenic mice with > 10 s of floating behavior in the retention test of the MWM no differences in spatial learning could be seen between Wt ($n = 10$) and CYP2C19Tg-Hem ($n = 3$) mice. Spatial learning was evaluated by measuring time to first platform crossing, number of total platform crossings and total time spent in the platform quadrant.

Furthermore, when exposing the same set of mice to the TST 3 days after the last session (retention test) of the MWM, the difference previously seen between the groups (no stress, Figure 5c) was attenuated, with a significantly reduced immobility time for the Wt compared with the CYP2C19Tg-Hem mice ($P = 0.0005$; MWM considered as stressor, Figure 5c). Notably, CYP2C19Tg-Hem mice displayed very similar immobility time in TST, independent on the exposure to prior MWM stress.

In the open-field test, evaluation of the total distance travelled over 30 min in the novel environment showed significantly increased locomotor activity in 15-week-old CYP2C19Tg-Hem mice compared with Wt mice ($P = 0.027$), which was already trending toward significance at 7 weeks of age ($P = 0.056$; Figures 5a and b). Moreover, at both ages, an increased response to the novel environment was found in CYP2C19Tg-Hem compared with Wt mice, as evident from increased distance travelled measured over the first 15 min (7 weeks: $P = 0.0048$; 15 weeks: $P = 0.0032$) with no difference in habituation between the genotypic groups (Figures 5a and b).

Adult CYP2C19Tg-Hem male mice subjected to the light-dark box test spent significantly less time in the exposed light compartment compared with Wt controls (41%; $P = 0.0024$; Figure 5d), thus suggesting an anxious phenotype. A similar trend was observed in 7-week-old mice ($P = 0.065$; Figure 5d).

Effect of acute restraint stress on serum CORT levels and expression of *c-fos* in the hippocampus

An independent set of mice that had not been exposed to behavioral testing was subjected to 30 min of acute restraint stress to assess any differences in stress response between genotype groups. CORT is the major stress hormone in rodents,³⁰ but no difference in serum CORT levels could be detected between CYP2C19Tg-Hem mice and Wt controls at either 7 or 15 weeks of age (Supplementary Figure 7). Although there was no difference in basal neuronal activation as measured by *c-fos* expression, at either age, *c-fos* mRNA levels were significantly higher after 60 min (30-min restraint stress + 30-min recovery) in CYP2C19Tg-Hem compared with Wt mice at both 7 and 15 weeks of age (51%, $P = 0.0030$ and 46%, $P = 0.026$, respectively; Figures 5e and f).

DISCUSSION

Previous studies have indicated that *CYP2C19* polymorphism affects personality traits^{31,32} as well as depressive symptoms in humans,⁹ thus suggesting that high or low *CYP2C19* enzyme activity might affect brain functions and influence the risk of developing psychiatric disorders. Thus, the primary aim of this study was to investigate if expression of the human *CYP2C19* gene in mice could influence brain function or animal behavior.

By analyzing mRNA levels in transgenic mouse brains, we found high *CYP2C19* expression in the developing brain, whereas the brain expression in 7-week-old mice was low, as has also been previously shown in the adult transgenic brain.²⁴ The developmental expression of *CYP2C19* in brain is particularly interesting since *CYP2C19* is one of only a few human CYP enzymes expressed in the human liver at relatively high levels during the prenatal period, thus suggesting an important developmental function.³³ Analysis of expression of *CYP2C19* in human fetal brain regions from three different donors showed that *CYP2C19* can be expressed at a level representing slightly $> 1/200$ of human adult liver, which is high taking into account that the P450 expression in liver is very high and present in 70% of all cells.

We observed extensive alterations in brain morphology of newborn CYP2C19Tg-Hem mice, including severely reduced hippocampal size and complete callosal agenesis. As a result of the lethality of the homozygous genotype, we carried out further investigations in CYP2C19Tg-Hem mice.

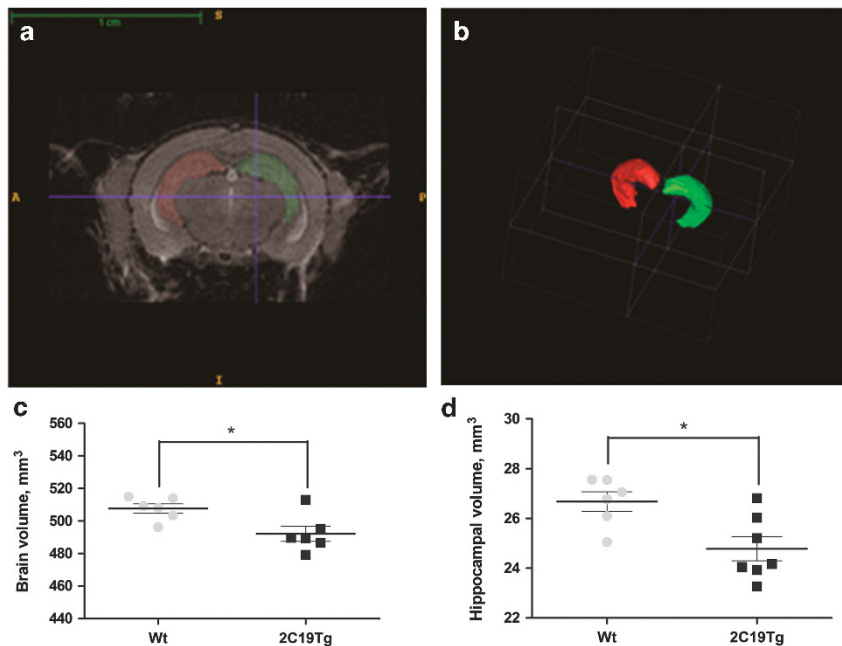


Figure 3. Brain and hippocampal volumes are decreased in adult CYP2C19Tg-Hem compared with wild-type (Wt) mice as determined by magnetic resonance imaging (MRI). Adult (15 weeks) CYP2C19Tg-Hem ($n = 7$) and Wt ($n = 7$) mice were examined using a 9.4 T Varian MRI system. Whole-brain and hippocampal volumes were manually outlined using the software ITK-SNAP. (a, b) Manual outlining of a representative Wt left (green) and right (red) hippocampus, the right image displays a three-dimensional illustration of the whole brain after outlining. (c) A mean 3.1% ($P = 0.018$) lower total whole-brain volume was observed in CYP2C19Tg-Hem compared with Wt mice. (d) Hippocampal volumes were more profoundly reduced than total brain volume with a mean reduction of 7.1% ($P = 0.013$) in CYP2C19Tg-Hem compared with Wt mice. Measurements from two mice had to be excluded because of poor positioning and thus incomplete field of view during the imaging. Data are presented as mean with s.e.m. * $P < 0.05$.

The hippocampal formation is one of the most studied limbic structures with a critical role in learning and memory,³⁴ stress adaptation and sensitivity,³⁵ and emotional processing.¹ Furthermore, hippocampal dysfunction is believed to have a major role in the pathophysiology of both MDD and anxiety-related disorders.^{1,2} We investigated hippocampal integrity in adult CYP2C19Tg-Hem mice (15-week-old) using area measurements of brain sections, and MRI on anesthetized animals. Both methods showed that the hippocampal formation was 7–11% smaller in CYP2C19Tg-Hem mice compared with Wt controls, whereas a 3.1% size reduction was seen in whole-brain volumes. As the hippocampus and whole-brain volumes did not correlate as measured by MRI, we did not normalize hippocampal volumes with whole-brain volumes. Corpus callosum integrity was not further investigated in adult mice. However, the callosal agenesis in CYP2C19Tg-Hem new-born pups further suggests that important, MDD-related brain functions, such as white matter integrity are affected in the transgenic mice during brain development.^{36–38}

We found that the hippocampal volume reduction was specific for the caudal-ventral part, and did not affect the whole hippocampal formation. Furthermore, the specific and severe malformation and 60% shrinkage of the hippocampus in CYP2C19Tg-Hem mice at PND0 also suggests a specific effect of CYP2C19 on this brain structure. As reviewed by Fanselow and Dong,³⁹ the caudal-ventral part of the hippocampus is highly implicated in the regulation of emotions and stress responses supporting this morphology finding in correlation to our behavioral data.^{39,40} The hippocampal area was also measured in sections from 7-week-old mice with a similar result, thus suggesting that the smaller hippocampal volume is established early in life, most probably during fetal life as based on the drastic changes in CYP2C19Tg-Hem mice at PND0. As mentioned, reduced hippocampal volumes have been reported in several psychiatric

disorders in humans. Hippocampal volume measurements have been most extensively studied in MDD patients with reductions ranging between 5 and 10%^{5,6,41} resembling effects observed in the adult CYP2C19Tg-Hem mice (7–11%).

The DG of the hippocampus is a neurogenic niche and adult neurogenesis is critical for hippocampal function.^{42,43} When investigating the number of immature DCX-positive neurons in adult (15-week-old) mice, we found a 42% reduction of this specific cell population in CYP2C19Tg-Hem mice. Besides being a step in the maturation process of new neurons, DCX-positive cells have unique electrophysiological properties, important for hippocampal signal processing. Immature neurons are highly excitable, display greater synaptic plasticity than mature neurons and long-term potentiation is more easily induced in this young neuron population.^{44,45} The large pool of DCX-positive cells in the DG is thought to be available for encoding new experiences^{46,47} and are furthermore suggested to be of importance in regulating stress reactivity both by negative feed-back on the hypothalamic–pituitary–adrenal-axis and in stress and depression-related behavioral tasks.³⁵ Since the proliferation rate in the DG, as measured by BrdU and Ki-67, was not affected at 7 or 15 weeks of age, the reduction of immature neurons appears to be specific, and could reflect a defect in the neuronal maturation process. GABAergic interneurons support the maturation of young neurons during development and in the adult hippocampus, thus suggesting that reduced GABAergic input to immature neurons in the DG could cause the reduction of DCX-positive cells.^{48,49} The reduced number of GABAergic interneurons in the DG could also influence overall hippocampal activity because of a reduced inhibition of incoming signals, consequently leading to a hyperactivated hippocampus.⁵⁰ As the hippocampus is highly involved in the response to stress,^{35,39} the reduction in hippocampal volume, as well as in DCX-positive cells in the DG of CYP2C19Tg-Hem mice, could implicate an altered stress response in these mice. In fact,

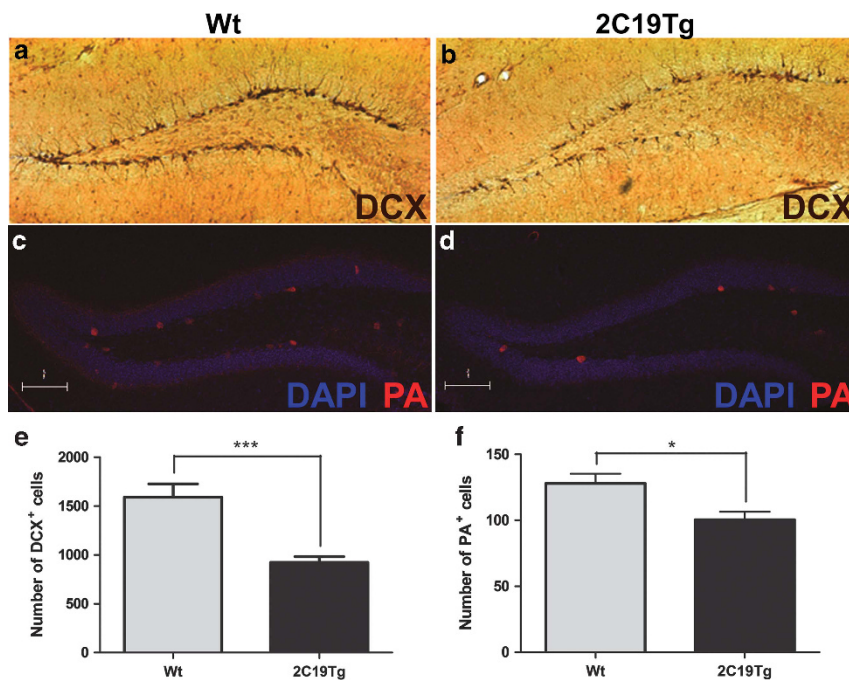


Figure 4. Reduced numbers of immature (double-cortin (DCX)-positive) neurons and GABAergic (parvalbumin (PA)-positive) interneurons in the hippocampal dentate gyrus (DG) of adult CYP2C19Tg-Hem compared with wild-type (Wt) mice. Distribution of immature DCX-positive neurons and PA-positive GABAergic interneurons in the DG of the hippocampus was addressed by immunohistochemistry in 15-week-old CYP2C19Tg-Hem and Wt mice. A total of eight coronal brain sections were analyzed from each mouse for an estimation of the total distribution of specific cells throughout the DG. **(a, b)** Representative images of the hippocampal DG with DCX-positive cells in brown. Whereas DCX neurons were smoothly lining the sub-granular zone of Wt mice, DCX neurons of CYP2C19Tg-Hem mice were frequently found integrated in the granular cell layer. **(c, d)** The hippocampal DG with representative images from PA stainings of 15-week-old CYP2C19Tg-Hem and Wt mice. 4,6-Diamidino-2-phenylindole (DAPI) is used as nuclear counter stain. **(e)** We observed a marked reduction in the total number of DCX-positive neurons in the DG of CYP2C19Tg-Hem ($n=9$) compared with Wt ($n=6$) mice that amounted to a 42% difference between genotypes ($P=0.0002$). **(f)** A 22% reduction ($P=0.017$) of the total number of PA-positive cells in the DG of CYP2C19Tg-Hem ($n=8$) compared with Wt ($n=5$) mice was found. No difference in the number of PA-positive cells in other hippocampal areas was found (data not shown). Data are presented as mean with s.e.m. * $P<0.05$; *** $P<0.001$.

although CORT levels did not differ between genotypes, we observed an increased neuronal activation, as measured by *c-fos* expression,^{51,52} in the hippocampus of CYP2C19Tg-Hem compared with Wt mice 30 min after acute restraint stress. Hence, the response and adaptation to stress in CYP2C19Tg mice hippocampi appear to be impaired.

Besides displaying an impaired stress response, CYP2C19 transgenic mice also exhibited a behavioral phenotype when exposed to a novel environment (open-field) or a short-term stressor (TST), both representing stressful stimuli. Indeed, CYP2C19Tg-Hem mice (both 7- and 15-week-old) displayed an increased reactivity to the open-field during the first 15 min of the test when the novelty stress is the highest. Furthermore, 15-week-old CYP2C19Tg-Hem mice showed a significantly shorter immobility time compared with Wt mice in the TST, which could be interpreted as increased stress sensitivity. As a matter of fact, exposure of the mice to a TST after the MWM revealed that Wt mice decreased their immobility time by 45% approaching the stable level of the transgenic mice. These data indicate that CYP2C19Tg-Hem mice are more stress sensitive, which furthermore also could be an explanation for the acquired despair (floating) behavior in response to 5 days of swim maze training. This despair phenotype resembles depressive-like behavior in the forced-swim test.^{53,54} In combination with increased stress sensitivity, CYP2C19 transgenic mice also displayed increased anxiety behavior in the light–dark box test. The comorbidity of generalized anxiety disorder and MDD is high, with environmental stressors and increased stress sensitivity being major risk factors for these disorders.^{55,56} The CYP2C19 transgenic mice display a

behavioral phenotype relevant with regards to CYP2C19s recent association with depressive symptoms in human subjects.⁹

An important question concerns by which mechanism the increased levels of CYP2C19 might cause the mouse phenotype and an obvious point is by metabolism of endogenous compounds. CYP2C19 has been shown to metabolize many different compounds including polyunsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid^{57,58} and several psychoactive sex hormones such as estradiol,¹² estrone,¹³ progesterone and testosterone.¹⁴ The effects of polyunsaturated fatty acids and steroid hormones on brain function have been extensively studied, and shown to affect not only embryonic development but also adolescent brain maturation.^{59–65} CYP2C19 has furthermore been shown to metabolize the exogenous cannabinoid cannabidiol,⁶⁶ as well as having a high affinity for cannabinoid-related compounds.⁶⁷ These findings support the possibility of an endogenous role for CYP2C19 in the metabolism of compounds influencing brain development, although the identity of such compounds requires further research.

Besides CYP2C19, also the polymorphic enzyme CYP2D6, metabolizes antidepressants and psychoactive compounds.⁶⁸ Recently, higher frequencies of subjects carrying multiple active CYP2D6 genes (ultrarapid metabolizers) have been found in suicide cases⁶⁹ and subjects with increased suicidal risk or suicidal behavior.^{70,71} Thus, classical drug-metabolizing enzymes do have the potential to be important modulators of brain function.

In conclusion, our data indicate that expression of the human CYP2C19 gene in transgenic mice causes developmental

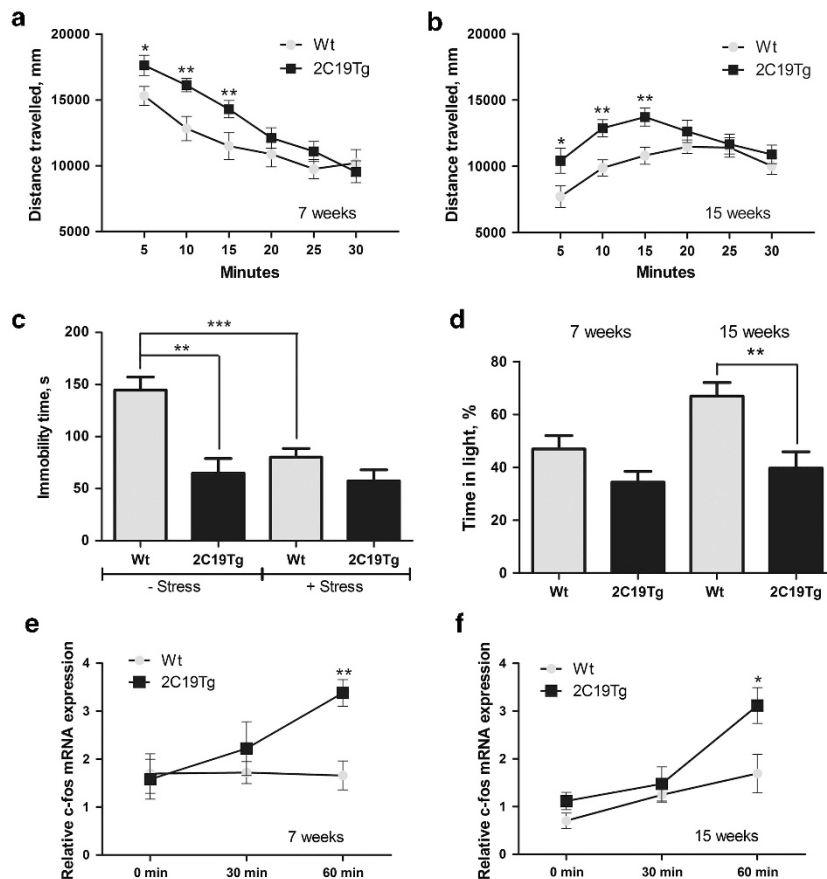


Figure 5. The behavioral phenotype of CYP2C19Tg-Hem mice is indicative of increased stress-sensitivity and anxiogenic-like behavior. **(a, b)** Mice were subjected to the open-field test for 30 min at both 7 and 15 weeks of age to investigate motor activity and reaction to a novel environment. Fifteen-week-old CYP2C19Tg-Hem mice displayed an increased total distance travelled compared with wild-type (Wt) controls ($P = 0.027$) with a similar trend at 7 weeks ($P = 0.056$; $n = 15$ mice per group at both ages; data not shown). Data are presented as distance travelled over time in 5-min bouts and the Student's *t*-test was performed on each bout. **(a)** Distance travelled over time in 7-week-old mice. CYP2C19Tg-Hem mice displayed increased motor activity compared with Wt controls during the first 15 min of the test ($P = 0.0048$). **(b)** At 15 weeks of age, CYP2C19Tg-Hem mice displayed the same increased reactivity to the novel environment as adolescent animals; CYP2C19Tg-Hem mice showed an increased total distance travelled compared with Wt mice during the first 15 min ($P = 0.0032$). The Student's *t*-test was performed on the total time travelled during the first three time points. **(c)** Mice were subjected to the tail-suspension test (TST) and immobility time was recorded during a 6-min session. There was no difference in immobility time between CYP2C19Tg-Hem and Wt mice at 7 weeks of age but a significant reduction in immobility time was recorded in 15-week-old CYP2C19Tg-Hem mice ($n = 10$) compared with controls ($n = 6$; 55%; $P = 0.0011$) here referred to as—stress. A separate group of mice were subjected to the TST after the Morris water maze (MWM), here regarded as a stressor (+ stress). Exposure of the mice to a TST after the MWM revealed that Wt mice ($n = 10$) decreased their immobility time by 45% down to the level of CYP2C19Tg-Hem mice ($n = 8$), whereas CYP2C19Tg-Hem mice displayed the same low immobility time with or without prior MWM stress. **(d)** CYP2C19Tg-Hem and Wt mice were subjected to the light–dark box test at 7 and 15 weeks of age. The time spent in the light and dark compartments was recorded during a test session of 5 min. At 15 weeks of age, CYP2C19Tg-Hem mice spent significantly less time in the light compartment than Wt mice (41% less; $P = 0.0024$, 15 mice per group), thus indicating an anxiogenic-like phenotype. At 7 weeks of age CYP2C19Tg-Hem mice displayed a trend toward spending less time in the light compartment ($P = 0.065$). **(e, f)** CYP2C19Tg-Hem and Wt mice at both 7 and 15 weeks of age were subjected to 30 min of acute restraint stress followed by immediate decapitation (30 min) or a 30-min recovery period in home cages before decapitation (60 min). Mice for baseline measurements were directly decapitated (0 min). Hippocampi were isolated for analysis of *c-fos* mRNA expression ($n = 5–6$ mice per group). CYP2C19Tg-Hem mice showed significantly higher *c-fos* mRNA levels than Wt mice at the 60-min time point at both ages, indicating increased neuronal activation in the hippocampus in response to acute stress. Data are presented as mean with s.e.m. * $P < 0.05$; ** $P < 0.01$.

alterations in the brain. This might be explained by CYP2C19-dependent metabolism of endogenous compounds leading to increased stress-sensitivity at behavioral, morphological and molecular levels, many of which are related to the altered hippocampal formation. The mechanistic basis for the alterations observed in the transgenic mouse model requires further studies that could lead to increased understanding of mechanisms and endogenous factors of importance for the development of hippocampus-related morphological and behavioral phenotypes. It can also be suggested that genetic *CYP2C19* polymorphism, which determines the presence or absence of the enzyme, is an important factor in determining the individual susceptibility to

stress- and anxiety-related disorders because of its influence on brain development. Such knowledge could be of importance for future drug-based interventions and the mouse model here presented used as a tool in drug discovery and development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Brain Foundation, Torsten och Ragnar Söderbergs Stiftelser, Vinnova, Knut and Alice Wallenberg Foundation and the

Swedish Research Council. The MRI studies were conducted at the Experimental MR Center at Karolinska Institutet and Karolinska Experimental Research and Imaging Center and we acknowledge their generous help. Human brain tissue was obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, USA. We appreciate support provided by Dr Martin Egeland, Dr Hans Wigzell and Dr Tomas Hökfelt.

REFERENCES

- Femenia T, Gomez-Galan M, Lindskog M, Magara S. Dysfunctional hippocampal activity affects emotion and cognition in mood disorders. *Brain Res* 2012; **1476**: 58–70.
- Kheirbek MA, Klemenhagen KC, Sahay A, Hen R. Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat Neurosci* 2012; **15**: 1613–1620.
- Woon FL, Hedges DW. Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. *Hippocampus* 2008; **18**: 729–736.
- Adriano F, Caltagirone C, Spalletta G. Hippocampal volume reduction in first-episode and chronic schizophrenia: a review and meta-analysis. *Neurosci* 2012; **18**: 180–200.
- Campbell S, Marriott M, Nahmias C, MacQueen GM. Lower hippocampal volume in patients suffering from depression: a meta-analysis. *Am J Psychiatry* 2004; **161**: 598–607.
- Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry* 2004; **161**: 1957–1966.
- Bell-McGinty S, Butters MA, Meltzer CC, Greer PJ, Reynolds 3rd CF, Becker JT. Brain morphometric abnormalities in geriatric depression: long-term neurobiological effects of illness duration. *Am J Psychiatry* 2002; **159**: 1424–1427.
- McKinnon MC, Yucel K, Nazarov A, MacQueen GM. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. *J Psychiatry Neurosci* 2009; **34**: 41–54.
- Sim SC, Nordin L, Andersson TM, Viriding S, Olsson M, Pedersen NL et al. Association between CYP2C19 polymorphism and depressive symptoms. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 1160–1166.
- Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 2007; **116**: 496–526.
- Fukasawa T, Suzuki A, Otani K. Effects of genetic polymorphism of cytochrome P450 enzymes on the pharmacokinetics of benzodiazepines. *J Clin Pharm Ther* 2007; **32**: 333–341.
- Cheng ZN, Shu Y, Liu ZQ, Wang LS, Ou-Yang DS, Zhou HH. Role of cytochrome P450 in estradiol metabolism in vitro. *Acta Pharmacologica Sinica* 2001; **22**: 148–154.
- Cribb AE, Knight MJ, Dryer D, Guernsey J, Hender K, Tesch M et al. Role of polymorphic human cytochrome P450 enzymes in estrone oxidation. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 551–558.
- Yamazaki H, Shimada T. Progesterone and testosterone hydroxylation by cytochromes P450 2C19, 2C9, and 3A4 in human liver microsomes. *Arch Biochem Biophys* 1997; **346**: 161–169.
- Sim SC, Risinger C, Dahl ML, Akillu E, Christensen M, Bertilsson L et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 2006; **79**: 103–113.
- Ragia G, Arvanitidis KI, Tavridou A, Manolopoulos VG. Need for reassessment of reported CYP2C19 allele frequencies in various populations in view of CYP2C19*17 discovery: the case of Greece. *Pharmacogenomics* 2009; **10**: 43–49.
- Kurzawski M, Gawronska-Szklarz B, Wrzesniewska J, Siuda A, Starzynska T, Drozdik M. Effect of CYP2C19*17 gene variant on Helicobacter pylori eradication in peptic ulcer patients. *Eur J Clin Pharmacol* 2006; **62**: 877–880.
- Martis S, Peter I, Hulot JS, Kornreich R, Desnick RJ, Scott SA. Multi-ethnic distribution of clinically relevant CYP2C genotypes and haplotypes. *Pharmacogenomics J* 2012; doi:10.1038/tpj.2012.10 (e-pub ahead of print).
- Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E. Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients. *Clin Pharmacol Ther* 2008; **83**: 322–327.
- Schenk PW, van Vliet M, Mathot RA, van Gelder T, Vulto AG, van Fessem MA et al. The CYP2C19*17 genotype is associated with lower imipramine plasma concentrations in a large group of depressed patients. *Pharmacogenomics J* 2010; **10**: 219–225.
- Huezo-Diaz P, Perroud N, Spencer EP, Smith R, Sim S, Viriding S et al. CYP2C19 genotype predicts steady state escitalopram concentration in GENDEP. *J Psychopharmacol* 2012; **26**: 398–407.
- Mrazek DA, Biernacka JM, O'Kane DJ, Black JL, Cunningham JM, Drews MS et al. CYP2C19 variation and citalopram response. *Pharmacogenet Genomics* 2011; **21**: 1–9.
- Lofgren S, Ekman S, Terelius Y, Fransson-Steen R. Few alterations in clinical pathology and histopathology observed in a CYP2C18&19 humanized mice model. *Acta Veterinaria Scandinavica* 2008; **50**: 47.
- Lofgren S, Baldwin RM, Hiratsuka M, Lindqvist A, Carlberg A, Sim SC et al. Generation of mice transgenic for human CYP2C18 and CYP2C19: characterization of the sexually dimorphic gene and enzyme expression. *Drug Metab Dispos* 2008; **36**: 955–962.
- Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *NeuroImage* 2006; **31**: 1116–1128.
- Landgren H, Curtis MA. Locating and labeling neural stem cells in the brain. *J Cell Physiol* 2011; **226**: 1–7.
- Lapple F, von Richter O, Fromm MF, Richter T, Thon KP, Wisser H et al. Differential expression and function of CYP2C isoforms in human intestine and liver. *Pharmacogenetics* 2003; **13**: 565–575.
- Gerbal-Chaloin S, Pascucci JM, Pichard-Garcia L, Daujat M, Waechter F, Fabre JM et al. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos* 2001; **29**: 242–251.
- Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* 2005; **29**: 571–625.
- Vreugdenhil E, de Kloet ER, Schaaf M, Datson NA. Genetic dissection of corticosterone receptor function in the rat hippocampus. *Eur Neuropsychopharmacol* 2001; **11**: 423–430.
- Ishii G, Suzuki A, Oshino S, Shiraishi H, Otani K. CYP2C19 polymorphism affects personality traits of Japanese females. *Neurosci Lett* 2007; **411**: 77–80.
- Yasui-Furukori N, Kaneda A, Iwashima K, Saito M, Nakagami T, Tsuchimine S et al. Association between cytochrome P450 (CYP) 2C19 polymorphisms and harm avoidance in Japanese. *Am J Med Genet B Neuropsychiatr Genet* 2007; **144B**: 724–727.
- Hines RN. Ontogeny of human hepatic cytochromes P450. *J Biochem Mol Toxicol* 2007; **21**: 169–175.
- Lewis S. Learning and memory: hippocampus plays multiple choice. *Nat Rev Neurosci* 2012; **13**: 600.
- Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 2011; **476**: 458–461.
- Liao Y, Huang X, Wu Q, Yang C, Kuang W, Du M et al. Is depression a disconnection syndrome? Meta-analysis of diffusion tensor imaging studies in patients with MDD. *J Psychiatry Neurosci* 2013; **38**: 49–56.
- Macmaster FP, Carrey N, Marie Langevin L. Corpus callosal morphology in early onset adolescent depression. *J Affect Disord* 2013; **145**: 256–259.
- Cole J, Chaddock CA, Farmer AE, Aitchison KJ, Simmons A, McGuffin P et al. White matter abnormalities and illness severity in major depressive disorder. *Br J Psychiatry* 2012; **201**: 33–39.
- Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 2010; **65**: 7–19.
- Kheirbek MA, Drew LJ, Burghardt NS, Costantini DO, Tannenholz L, Ahmari SE et al. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron* 2013; **77**: 955–968.
- Arnold D, McIntosh AM, Ebmeier KP, Munafò MR, Anderson IM. Magnetic resonance imaging studies in unipolar depression: systematic review and meta-regression analyses. *Eur Neuropsychopharmacol* 2012; **22**: 1–16.
- Snyder JS, Cameron HA. Could adult hippocampal neurogenesis be relevant for human behavior? *Behav Brain Res* 2012; **227**: 384–390.
- Song J, Christian KM, Ming GL, Song H. Modification of hippocampal circuitry by adult neurogenesis. *Dev Neurobiol* 2012; **72**: 1032–1043.
- Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* 2004; **429**: 184–187.
- Mongiati LA, Esposito MS, Lombardi G, Schinder AF. Reliable activation of immature neurons in the adult hippocampus. *PLoS One* 2009; **4**: e5320.
- Wiskott L, Rasch MJ, Kempermann G. A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus. *Hippocampus* 2006; **16**: 329–343.
- Keith JR, Priestler C, Ferguson M, Salling M, Hancock A. Persistent increases in the pool of doublecortin-expressing neurons in the hippocampus following spatial navigation training. *Behav Brain Res* 2008; **188**: 391–397.
- Masiulis I, Yun S, Eisch AJ. The interesting interplay between interneurons and adult hippocampal neurogenesis. *Mol Neurobiol* 2011; **44**: 287–302.
- Kullmann DM. Interneuron networks in the hippocampus. *Curr Opin Neurobiol* 2011; **21**: 709–716.
- Lodge DJ, Grace AA. Hippocampal dysfunction and disruption of dopamine system regulation in an animal model of schizophrenia. *Neurotoxicity Res* 2008; **14**: 97–104.

- 51 Okuno H. Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers. *Neurosci Res* 2011; **69**: 175–186.
- 52 Hansson AC, Fuxe K. Time-course of immediate early gene expression in hippocampal subregions of adrenalectomized rats after acute corticosterone challenge. *Brain Res* 2008; **1215**: 1–10.
- 53 Duman CH. Models of depression. *Vitam Horm* 2010; **82**: 1–21.
- 54 Swiergiel AH, Leskov IL, Dunn AJ. Effects of chronic and acute stressors and CRF on depression-like behavior in mice. *Behav Brain Res* 2008; **186**: 32–40.
- 55 Hoge EA, Ivkovic A, Fricchione GL. Generalized anxiety disorder: diagnosis and treatment. *BMJ* 2012; **345**: e7500.
- 56 de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005; **6**: 463–475.
- 57 Yao HT, Chang YW, Lan SJ, Chen CT, Hsu JT, Yeh TK. The inhibitory effect of polyunsaturated fatty acids on human CYP enzymes. *Life Sci* 2006; **79**: 2432–2440.
- 58 Fer M, Dreano Y, Lucas D, Corcos L, Salaun JP, Berthou F *et al*. Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. *Arch Biochem Biophys* 2008; **471**: 116–125.
- 59 Luchtman DW, Song C. Cognitive enhancement by omega-3 fatty acids from child-hood to old age: findings from animal and clinical studies. *Neuropharmacology* 2013; **64**: 550–565.
- 60 Cooke BM, Woolley CS. Gonadal hormone modulation of dendrites in the mammalian CNS. *J Neurobiol* 2005; **64**: 34–46.
- 61 Sa SI, Lukyanova E, Madeira MD. Effects of estrogens and progesterone on the synaptic organization of the hypothalamic ventromedial nucleus. *Neuroscience* 2009; **162**: 307–316.
- 62 Peper JS, van den Heuvel MP, Mandl RC, Hulshoff Pol HE, van Honk J. Sex steroids and connectivity in the human brain: a review of neuroimaging studies. *Psychoneuroendocrinology* 2011; **36**: 1101–1113.
- 63 Westberg L, Eriksson E. Sex steroid-related candidate genes in psychiatric disorders. *J Psychiatry Neurosci* 2008; **33**: 319–330.
- 64 He C, Qu X, Cui L, Wang J, Kang JX. Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. *Proc Natl Acad Sci USA* 2009; **106**: 11370–11375.
- 65 Brown GR, Spencer KA. Steroid hormones, stress and the adolescent brain: A comparative perspective. *Neuroscience* 2012; **50306-4522**: 01197–01199.
- 66 Jiang R, Yamaori S, Takeda S, Yamamoto I, Watanabe K. Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life Sci* 2011; **89**: 165–170.
- 67 Jiang R, Yamaori S, Okamoto Y, Yamamoto I, Watanabe K. Cannabidiol is a Potent Inhibitor of the Catalytic Activity of Cytochrome P450 2C19. *Drug Metab Pharmacokinetics* 2013; doi:10.2133/dmpk.DMPK-12-RG-129 (e-pub ahead of print).
- 68 Dorado P, Penas-Lledo EM, Llerena A. CYP2D6 polymorphism: implications for antipsychotic drug response, schizophrenia and personality traits. *Pharmacogenomics* 2007; **8**: 1597–1608.
- 69 Zackrisson AL, Lindblom B, Ahlner J. High frequency of occurrence of CYP2D6 gene duplication/multiduplication indicating ultrarapid metabolism among suicide cases. *Clin Pharmacol Ther* 2010; **88**: 354–359.
- 70 Stingl JC, Viviani R. CYP2D6 in the brain: impact on suicidality. *Clin Pharmacol Ther* 2011; **89**: 352–353.
- 71 Penas-Lledo EM, Dorado P, Aguera Z, Gratacos M, Estivill X, Fernandez-Aranda F *et al*. High risk of lifetime history of suicide attempts among CYP2D6 ultrarapid metabolizers with eating disorders. *Mol Psychiatry* 2011; **16**: 691–692.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>

Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)