Molecular Neurobiology

ISSN 0893–7648/07/35(1): 45–53/$30.00
ISSN (Online) 1559–1182

Developmental Role of Tryptophan Hydroxylase in the Nervous System

Kazuhiro Nakamura[1,*] and Hiroyuki Hasegawa[2]

[1]Department of Pathology, Juntendo University School of Medicine, Tokyo, Japan and [2]Department of Biosciences, Teikyo University of Science and Technology, Uenohara, Yamanashi, Japan

Abstract

The serotonin 5-hydroxytryptamine (5-HT) neurotransmitter system contributes to various physiological and pathological conditions. 5-HT is the first neurotransmitter for which a developmental role was suspected. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting reaction in the biosynthesis of 5-HT. Both TPH1 and TPH2 have tryptophan hydroxylating activity. TPH2 is abundant in the brain, whereas TPH1 is mainly expressed in the pineal gland and the periphery. However, TPH1 was found to be expressed predominantly during the late developmental stage in the brain. Recent advances have shed light on the kinetic properties of each TPH isoform. TPH1 showed greater affinity for tryptophan and stronger enzymic activity than TPH2 under conditions reflecting those in the developing brain stem. Transient alterations in 5-HT homeostasis during development modify the fine wiring of brain connections and cause permanent changes to adult behavior. An increasing body of evidence suggests the involvement of developmental brain disturbances in psychiatric disorders. These findings have revived a long-standing interest in the developmental role of 5-HT-related molecules. This article summarizes our understanding of the kinetics and possible neuronal functions of each TPH during development and in the adult.

Index Entries: Brain function; development; serotonin; tryptophan hydroxylase 1; tryptophan hydroxylase 2.

Introduction

Tryptophan hydroxylase (TPH, EC 1.14.16.4), a member of a family of pterin-dependent aromatic amino acid hydroxylases *(1)*, catalyzes the conversion of *L*-tryptophan to 5-hydroxy-*L*-tryptophan. This reaction is the initial and rate-limiting step in the biosynthesis of 5-hydroxytryptamine (5-HT) *(2–5)*. TPH has been extensively purified from various sources such as bovine pineal gland *(6)*, mouse mastocytoma *(7,8)*, and mammalian brains *(9–11)*.

Received July 5, 2006; Accepted September 8, 2006.
*Author to whom correspondence and reprint requests should be addressed. E-mail: kaz@med.juntendo.ac.jp

Physicochemical, enzymic, and immunochemical properties differ between TPHs of neural and non-neural tissue origin, and it has been accepted that neural TPH might be a different entity from the non-neural enzyme *(8,10,12, 13)*. The molecular basis of the differences between the neural and non-neural enzymes was only recently explained. Walther and colleagues *(14)* clearly demonstrated the distinction between the two types of TPHs and also found TPH2, a new TPH isoform. By gene targeting, they functionally ablated original *TPH1* gene in mice. Although the resulting animals were deficient for 5-HT in the periphery and in the pineal gland, they exhibited close to normal levels of 5-HT in the brain. This led them to the detection of a second TPH gene known as *TPH2 (14)*. The tryptophan hydroxylating activity of TPH2 was then demonstrated *(14,15)*. TPH2 has been identified and cloned in human, mice, rat, chicken, zebrafish, torafugu, and fruit fly *(14,16,17)*. Therefore, TPH2 has been distinguished from the well-characterized original TPH1. Human TPH1 is located on chromosome 11, whereas TPH2 is on chromosome 12. Human TPH1 and TPH2 display 72% sequence homology and have high sequence identity within the C-terminal catalytic domain. All residues that have been detected to be important for the structural and functional properties of TPH1 are conserved in TPH2. However, TPH2 has a divergent N-terminal regulatory domain, which shows more homology to that of tyrosine hydroxylase *(18)*.

5-HT is a major modulator of behavior in vertebrates and invertebrates, and deficiencies in the serotonergic system account for several behavioral disorders in humans. The serotonergic central neurons produce their effects through secretion from synaptic terminals, acting locally in hard-wired circuits, and occasionally from extrasynaptic axonal and somatodendritic release sites in the absence of postsynaptic targets, producing paracrine effects. Because 5-HT has been implicated in various pathological functions in central nervous systems *(19,20)*, 5-HT is a major therapeutic target in patients with psychiatric disorders. Numerous studies have suggested associations between various neuropsychiatric disorders and genes that modulate central serotonergic neurotransmission, such as the 5-HT transporter (SERT; ref. *21*), 5-HT receptors *(19)*, and monoamine oxidases *(22)*. Therefore, the brain 5-HT system is a major target in the treatment of several psychiatric disorders that use agents such as tricyclic antidepressants, selective serotonin re-uptake inhibitors, monoamine oxidase inhibitors, and psychostimulants *(19,23)*.

In addition to its action as a crucial neurotransmitter in various regions of the adult brain, 5-HT is essential for the development of 5-HT neurons in an autocrine manner *(24)*. Therefore, changes in brain 5-HT content during development could disturb the development of 5-HT neurons, which might cause permanent changes in adult behavior. *TPH2* messenger RNA (mRNA) is preferentially expressed in the brain *(14)* and in the peripheral myenteric neurons in the gut *(25)*. Conversely, *TPH1* mRNA is mainly expressed in the pineal gland and the periphery *(14)*. However, *TPH1* was found to be expressed predominantly during the late developmental stage in the brain *(26)*. Consistently, *TPH1* gene has been the subject of intensive study regarding its possible involvement in many psychiatric and behavioral traits *(27–31)*.

In this review article, we initially discuss the kinetic properties of each TPH, followed by the possible involvement of each TPH in behavioral traits and psychiatric disorders.

Kinetic Properties of Tryptophan Hydroxylases

Hydroxylation of an aromatic ring is a fundamental reaction in biology. TPH catalyzes the formation of 5-hydroxy-*L*-tryptophan (5-HTP) from *L*-tryptophan, the first step in the biosynthesis of the neurotransmitter 5-HT. Aromatic *L*-amino-acid decarboxylase (AADC) subsequently mediates the production of 5-HT. Although the lack of a pure enzyme has

complicated elucidation of the regulatory properties of TPH, an accumulating body of evidence has clarified the factors that regulate TPH activity. First, in the presence of sulfhydryl compounds, such as dithiothreitol and mercaptoethanol, ferrous iron dramatically activates TPH in vitro. TPH in fresh extracts is usually very unstable and converts to an inactive form, whose activity can be restored far beyond initial levels by incubation with Fe^{2+} in the presence of dithiothreitol *(32–36)*. TPH of peripheral sources is fully active at concentrations of Fe^{2+} higher than 1×10^{-8} *M* but has no activity at concentrations lower than 2×10^{-12} *M*. Therefore, the activation of the enzyme could be considered in terms of ferrous iron activation *(37)*. Second, the activity of the enzyme is affected by phosphorylation. Ehret et al. *(38)* demonstrated the direct phosphorylation of TPH by CaMKII. Additionally, protein kinase A (PKA) has been shown to phosphorylate this enzyme, resulting in a twofold activation if the activator protein is present *(39)*. Third, the enzyme level in the cell is maintained by rapid turnover driven by ubiquitine-proteasome system (*40*,*41*). The ubiquitination of TPH is regulated by phosphorylation of the enzyme *(42)*. The effects of these factors on the enzymatic activities were described using various sources before the demonstration of the new enzyme TPH2; therefore, they have not been unequivocally compared between TPH1 and TPH2.

A minimal kinetic mechanism for a hydroxylase involves substrate binding, formation of the hydroxylating intermediate, hydroxylation, and product dissociation. TPH activity also depends on the cosubstrate. TPH is a mono-oxygenase, incorporating one atom of oxygen from molecular oxygen into the substrate and reducing the other atom to water. The two electrons required for the reduction of the second atom to water are supplied by the 6R-*L-erythro*-5,6,7,8-tetrahydrobiopterin (BH4). BH4 acts as cosubstrate rather than as a tightly bound cofactor.

The differences in the activities of TPH1 and TPH2 under various concentrations of BH4 were reported. McKinney et al. *(43)* compared the kinetic properties of TPH1 and TPH2 using human TPH1 and TPH2 fusion proteins derived from *Escherichia coli* and an in vitro transcription/translation system *(43)*. They found different kinetic properties between the two enzymes at various concentrations of tryptophan and BH4. In our study, histidine hexamer-tagged mouse TPH1 and TPH2 proteins were expressed in Cos7 cells, and the dependence of the activities of TPH1 and TPH2 on concentrations of the substrate tryptophan and the cosubstrate BH4 were compared to the respective cell extracts using His-tag expression as the reference (ref. *26*; Fig. 1). Apparent Kms for tryptophan at 400 μ*M* of BH4 were 16.6 and 19.2 μ*M* in TPH1 and TPH2, respectively. However, the affinity to tryptophan was much greater in TPH1 at a lower concentration of BH4; the Km was 7.5 μ*M* for tryptophan at 4 μ*M* of BH4, whereas that of TPH2 was constant (19.2 μ*M*), suggesting that TPH1 sustains enzymic activity even with lowered tryptophan levels under possible BH4-limiting conditions.

Because *TPH1* was found to be expressed mostly during the developmental stage in the brain *(26)*, our group extended the analysis to conditions mimicking the brain stem of the P21 mouse. The concentration of BH4 in the brain stem of various mouse strains at P21was around 0.9 nmol/g. Assuming an even distribution of BH4 in the tissue, around 1 μ*M* could be estimated, which was far less than the Kms of both TPH1 and TPH2 for BH4 (around 20 μ*M*). Tryptophan concentrations in mice at P21 were 15 to 30 nmol/g of wet tissue, which are around the Km level and far less than substrate saturation. Therefore, TPH activity depends quasilinearly on the concentration of tissue tryptophan. Importantly, the comparison of kinetics between TPH1 and TPH2 was made under conditions reflecting those in the developing brain stem of P21 mouse—that is, a low BH4 concentration (less than 4 μ*M*). In such conditions, TPH1 uses tryptophan to synthesize 5-HT more efficiently than TPH2. In this context, TPH1 exerts its potential activity better with respect to tryptophan dependence.

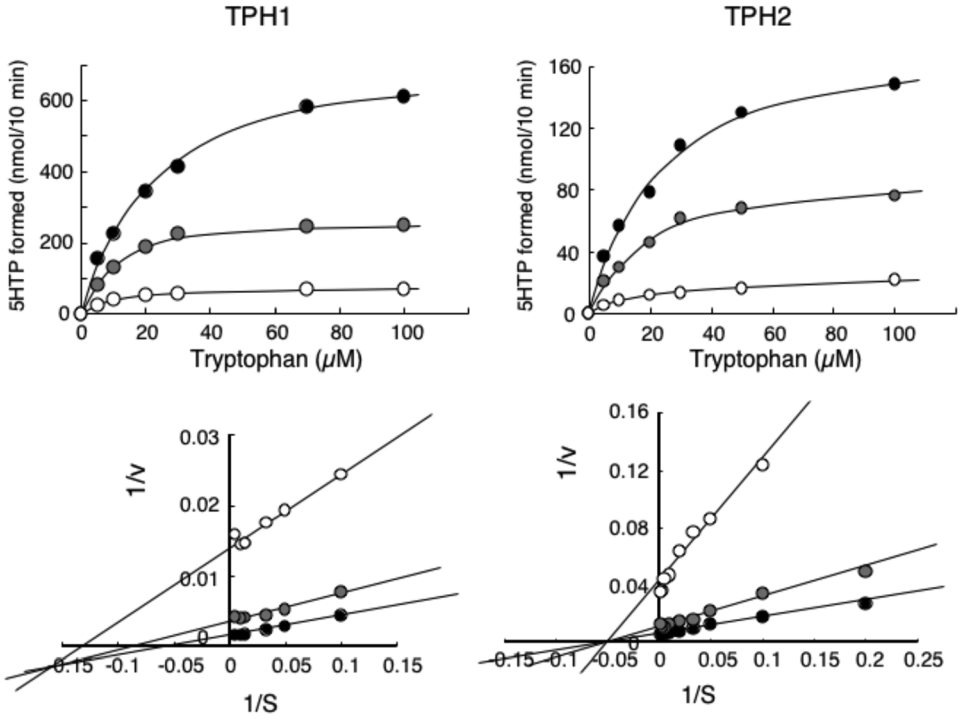

Fig. 1. Comparison of kinetics between TPH1 and TPH2. Upper panels are Michaelis-Menten plots of tryptophan hydroxylase activity at various concentrations of tryptophan and BH4 (4 μ*M*/open circle; 40 μ*M*/gray circle, and 400 μ*M*/closed circle). Lower panels are corresponding double reciprocal plots. (ref. *26*; copyright 2006 by the Society for Neuroscience).

Therefore, TPH1 is likely responsible for maintaining 5-HT levels in the brain under such conditions during late developmental stage. Indeed, two mouse strains (NZW and SWR) with weak *TPH1* expression showed low brain 5-HT levels only during development in vivo *(26)*.

Involvement of Tryptophan Hydroxylases in Psychiatric Conditions and Behavioral Traits

5-HT neurons arising from serotonergic cell body groups in the dorsal and median raphe nuclei in the midbrain form a dense plexus of axonal processes projecting to several areas such as the cortex and limbic system. These regions are responsible for the generation and expression of motivational and affective states. Serotonergic neurotransmission is described as paracrine or volume transmission, and therefore, 5-HT is believed to play a neuromodulatory role in these regions.

The human *TPH1* gene represents a candidate for one of the genes involved in psychiatric conditions. The polymorphism A779C in intron 7 of *TPH1* has been reported to be associated with alcoholic offenders *(28)*. Additionally, serotonergic dysfunction appears to be greatest in subjects who have attempted or completed suicide using violent means. The association of *TPH1* with suicidal behavior has been well-studied. For example, A779C has been reported to be associated with suicidal behavior in depressed patients *(27)*. Another study reported that the association between

TPH1 and suicidal behavior was stronger with violent suicidal behavior *(44)*.

Several recent meta-analyses have concluded that there is an association between A218 and a history of suicide attempts in Caucasians *(30,45)*. Suicidal behavior is implicated in the relationship between *TPH1* and mood disorder. Bellivier et al. *(46)* initially reported an association between *TPH1* and bipolar disorder. However, this study showed that the frequency of the allele was significantly associated with the history of suicidal behavior in bipolar subjects. Therefore, the reported association with bipolar disorder might be linked to suicidal behavior. Four subsequent studies found no association between *TPH1* and bipolar disorder, and three of these studies, in which the population of patients with bipolar disorder was stratified according to their history of suicidal behavior, found no association with suicidal behavior in subjects with bipolar disorder *(47–50)*. Therefore, *TPH1* may be involved in susceptibility to suicidal behavior in some populations. Courtet et al. *(51)* suggested that TPH may be a quantitative risk factor where the more pronounced the serotonergic dysfunction, the higher the levels of anger, and the more severe the suicidal act (lethal, violent, repeated suicide attempts, completed suicide).

A functional (C1473G) single-nucleotide polymorphism in the mouse *TPH2* gene that results in the substitution of Pro447 with Arg447 leads to decreased 5-HT levels in PC12 cells *(15)*. In BALB/c and DBA/2 mice that are homozygous for the 1473G allele, brain 5-HT tissue content and synthesis are reduced. The contributions of TPH2 to behavioral traits were examined in mice using strain differences. Cervo et al. *(52)* studied strain differences in the response to citalopram, a selective 5-HT reuptake inhibitor. Citalopram reduced immobility time in the forced swimming test, a mouse model used to assess the antidepressant potential of drugs, in C57BL/6J and 129/Sv mice but had no such effect in DBA/2 and BALB/c mice that showed less synthesis of brain 5-HT. However, DBA/2 and BALB/c mice were responsive to citalopram in the tail suspension test, another behavioral test for animal depression *(53)*. Additionally, there are reports suggesting an association between *TPH2* and psychiatric conditions in patients. Further studies will confirm an involvement of TPH2 in each psychiatric condition.

Developmental Role of TPH1

Neurotransmitters modulate the construction and plasticity of brain circuits. Serotonergic neurons are among the earliest neurons to be generated, and 5-HT is released by growing axons before conventional synapses are established. Pharmacological studies initially showed that 5-HT can modulate numerous developmental events, including cell division, neuronal migration, cell differentiation, and synaptogenesis *(54–58)*. Additionally, numerous neurons transiently express partial serotonergic phenotypes during development. These neurons store and release 5-HT but cannot synthesize it. Additionally, numerous 5-HT receptors show early and dynamic expression during development. Therefore, 5-HT was suspected to be a neurotransmitter involved in neuronal development. During the generation of serotonergic neurons early in development, several transcription factors could directly activate the transcription of the genes that define the 5-HT phenotype: *TPH*, *AADC*, *SERT* and the vesicular monoamine transporter gene *(59,60)*. As the raphe neurons begin to differentiate, 5-HT is released and could have a trophic autocrine effect. In cultures of raphe neurons, 5-HT amplifies its own synthesis and increases axon outgrowth *(61–63)*. Altered 5-HT levels during development affect adult behavior. The abnormalities in open-field exploration and beam-walking of mice deprived of monoamine oxidase A, the main enzyme responsible for the degradation of 5-HT, were rescued when 5-HT levels were controlled during early postnatal life *(64)*.

We demonstrated the effect of weak *TPH1* expression in the developing brain on adult behavior in mice *(26)*. NZW and SWR mice,

but not NZB mice, had functional polymorphisms in the promoter and 3′ untranslated regions of the *TPH1* gene that resulted in low 5-HT levels during development but not in the adult *(26)*. We observed a longer immobility time in adult NZW and SWR mice compared to NZB mice in forced swim and tail suspension tests *(26)*. Because the effect was more apparent in NZW mice than SWR mice in the tail suspension test, the molecules other than TPH1 also contributed to the performances of these mice. Paroxetine, a serotonin-specific reuptake inhibitor, shortened the immobility time in forced swim tests in adult control mice, whereas paroxetine was ineffective in NZW mice. Although imipramine treatment partially decreased immobility in NZW mice, the immobility time was still longer than that of NZB mice. These observations suggest that functional alterations in the 5-HT system induced by low 5-HT levels during development cannot be fully overcome in NZW mice. TPH has attracted a great deal of scientific interest because it catalyzes reactions critical for the proper functioning of the central nervous system. *TPH1* expression could not be detected using reverse transcriptase-polymerase chain reaction at P7. Instead, *TPH1* was expressed at a later developmental stage *(26)*. Therefore, TPH1 is likely involved in the fine-tuning or maturation of 5-HT neurons, which might cause permanent changes in adult behavior. On the other hand, it is possible that TPH2 is involved in psychiatric disorders through regulation of 5-HT neuronal function in the adult brain, considering that TPH2 is the dominant enzyme abundant in the brain. The temporally distinct expression pattern of TPH1 and TPH2 might enable elaborate regulation of 5-HT levels during developmental and adult stages.

Conclusion

The formation of proper neural circuits is sensitive to activities during well-defined intervals of postnatal development, and 5-HT is essential for the development of 5-HT neurons in an autocrine manner in addition to its action as a crucial neurotransmitter in various regions of the adult brain. Proper TPH content during development is likely required for the maturation of adult brain functions. Further enzymatic studies are needed under conditions in several brain regions at various developmental stages. The polymorphisms in the *TPH2* gene have been examined in several psychiatric disorders. Additionally, considering the results obtained with neurodevelopmental models of psychiatric disorders *(65)*, a relationship between the polymorphisms in the *TPH1* gene and functional neurodevelopmental abnormalities might be considered in patients with psychiatric disorders.

References

1. Kaufman S. and Fisher D. B. (1974) Pterin-dependent aromatic amino acid hydroxylases. In: Hayaishi O., ed., Molecular Mechanisms of Oxygen Activation, New York and London: Academic Press, pp. 285–369.
2. Hosoda S. and Glick D. (1965) Biosynthesis of 5-hydroxytryptophan and 5-hydroxytryptamine from tryptophan by neoplastic mouse mast cells. *Biochim. Biophys. Acta.* **111,** 67–78.
3. Grahame S. D. (1967) The biosynthesis of 5-hydroxytryptamine in brain. *Biochem. J.* **105,** 351–360.
4. Lovenberg W., Jequier E., and Sjoerdsma A. (1967) Tryptophan hydroxylation: measurement in pineal gland, brainstem, and carcinoid tumor. *Science* **155,** 217–219.
5. Ichiyama A., Nakamura S., Nishizuka Y., and Hayaishi O. (1970) Enzymic studies on the biosynthesis of serotonin in mammalian brain. *J. Biol. Chem.* **245,** 1699–1709.
6. Ichiyama A., Hasegawa H., Tohyama C., Dohmoto C., and Kataoka T. (1976) Some properties of bovine pineal tryptophan hydroxylase. *Adv. Exp. Med. Biol.* **74,** 103–117.
7. Hosoda S. (1975) Further studies on tryptophan hydroxylase from neoplastic murine mast cells. *Biochim. Biophys. Acta.* **397,** 58–68.
8. Nakata H. and Fujisawa H. (1982) Tryptophan 5-monooxygenase from mouse mastocytoma P815. A simple purification and general properties. *Eur. J. Biochem.* **124,** 595–601.

9. Tong J. H. and Kaufman S. (1975) Tryptophan hydroxylase. Purification and some properties of the enzyme from rabbit hindbrain. *J. Biol. Chem.* **250,** 4152–4158.
10. Nakata H. and Fujisawa H. (1982) Purification and properties of tryptophan 5-monooxygenase from rat brain-stem. *Eur. J. Biochem.* **122,** 41–47.
11. Cash C. D., Vayer P., Mandel P., and Maitre M. (1985) Tryptophan 5-hydroxylase. Rapid purification from whole rat brain and production of a specific antiserum. *Eur. J. Biochem.* **149,** 239–245.
12. Kuhn D. M., Meyer M. A., and Lovenberg W. (1980) Comparisons of tryptophan hydroxylase from a malignant murine mast cell tumor and rat mesencephalic tegmentum. *Arch. Biochem. Biophys.* **199,** 355–361.
13. Hasegawa H., Yanagisawa M., Inoue F., Yanaihara N., and Ichiyama A. (1987) Demonstration of non-neural tryptophan 5-mono-oxygenase in mouse intestinal mucosa. *Biochem. J.* **248,** 501–509.
14. Walther D. J., Peter J. U., Bashammakh S., et al. (2003) Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* **299,** 76.
15. Zhang X., Beaulieu J. M., Sotnikova T. D., Gainetdinov R. R., and Caron M. G. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science **305,** 217.
16. Teraoka H., Russell C., Regan J., et al. (2004) Hedgehog and Fgf signaling pathways regulate the development of tphR-expressing serotonergic raphe neurons in zebrafish embryos. *J. Neurobiol*. **60,** 275–288.
17. Coleman C. M. and Neckameyer W. S. (2005) Serotonin synthesis by two distinct enzymes in Drosophila melanogaster. *Arch. Insect Biochem. Physiol*. **59,** 12–31.
18. McKinney J., Knappskog P. M., Pereira J., et al. (2004) Expression and purification of human tryptophan hydroxylase from *Escherichia coli* and *Pichia pastoris*.
19. Lucki I (1998) The spectrum of behaviors influenced by serotonin. *Biol. Psychiatry* **44,** 151–162.
20. Lesch K. P. (2004) Gene-environment interaction and the genetics of depression. *J. Psychiatry Neurosci.* **29,** 174–184.
21. Lesch K. P., Bengel D., Heils A., et al. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274,** 1527–1531.
22. Shih J. C., Chen K., and Ridd M. J. (1999) Monoamine oxidase: from genes to behavior. *Annu. Rev. Neurosci.* **22,** 197–217.
23. Gainetdinov R. R. and Caron M. G. (2003) Monoamine transporters: From genes to behavior. *Annu. Rev. Pharmacol. Toxicol.* **43,** 261–284.
24. Gaspar P., Cases O., and Maroteaux L. (2003) The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* **4,** 1002–1012.
25. Côté F., Thevenot E., Fligny C., et al. (2003) Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc. Natl. Acad. Sci. U.S.A.* **100,** 13,525–13,530.
26. Nakamura K., Sugawara Y., and Sawabe K., et al. (2006) Late developmental stage-specific role of tryptophan hydroxylase 1 in brain serotonin levels. *J. Neurosci.* **26,** 530–534.
27. Mann J. J., Malone K. M., Nielsen D. A., Goldman D., Erdos J., and Gelernter J. (1997) Possible association of a polymorphism of the tryptophan hydroxylase gene with suicidal behavior in depressed patients. *Am. J. Psychiatry* **154,** 1451–1453.
28. Nielsen D. A., Virkkunen M., Lappalainen J., et al. (1998) A tryptophan hydroxylase gene marker for suicidality and alcoholism. *Arch. Gen. Psychiatry* **55,** 593–602.
29. Rujescu D., Giegling I., Sato T., Hartmann A. M., and Moller H. J. (2003) Genetic variations in tryptophan hydroxylase in suicidal behavior: analysis and meta-analysis. *Biol. Psychiatry* **54,** 465–473.
30. Bellivier F., Chaste P., and Malafosse A. (2004) Association between the TPH gene A218C polymorphism and suicidal behavior: a meta-analysis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **124,** 87–91.
31. Peters E. J., Slager S. L., McGrath P. J., Knowles J. A., and Hamilton S. P. (2004) Investigation of serotonin-related genes in antidepressant response. *Mol. Psychiatry* **9,** 879–889.
32. Ichiyama A., Hori S., Mashimo Y., Nukiwa T., and Makuuchi H. (1974) The activation of bovine pineal tryptophan 5-monooxygenase. *FEBS Lett.* **40,** 88–91.
33. Ichiyama A., Hasegawa H., Tohyama C., Dohmoto C., and Kataoka T. (1976) Some properties of bovine pineal tryptophan hydroxylase. *Adv. Exp. Med. Biol.* **74,** 103–117.
34. Inoue F., Hasegawa H., Yamada M., and Ichiyama A. (1987) The serotonin content and tryptophan 5-monooxygemase activity in the stomach of an athymic mouse and mast cell-deficient mouse. *Biomed. Res.* **8,** 53–59.

35. Hasegawa H., Yanagisawa M., and Ichiyama A. (1983) Three discrete activity states of mastocytoma tryptophan 5-monooxygenase. In: Nozaki M., Yamamoto S., Ishimura Y., Coon M. J., Ernster L., and Estabrook R.W., eds., Oxygenases and Oxygen Metabolism, London, UK: Academic Press, pp. 296–304.
36. Yanagisawa M., Hasegawa H., Ichiyama A., Hosoda S., and Nakamura W. (1984) Comparison of serotonin-producing murine mastocytomas, P-815 and FMA3: Determination of tryptophan hydroxylase, aromatic L-amino acid decarboxylase, and cellular concentration of tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Biomed. Res.* **5,** 19–28.
37. Hasegawa H. and Ichiyama A. (2005) Distinctive iron requirement of tryptophan 5-monooxygenase: TPH1 requires dissociable ferrous iron. *Biochem. Biophys. Res. Commun.* **338,** 277–284.
38. Ehret M., Cash C. D., Hamon M., and Maitre M. (1989) Formal demonstration of the phosphorylation of rat brain tryptophan hydroxylase by Ca2+/calmodulin-dependent protein kinase. *J. Neurochem.* **52,** 1886–1891.
39. Makita Y., Okuno S., and Fujisawa H. (1990) Involvement of activator protein in the activation of tryptophan hydroxylase by cAMP-dependent protein kinase. *FEBS Lett.* **268,** 185-188.
40. Hasegawa H., Kojima M., Oguro K., and Nakanishi N. (1995) Rapid turnover of tryptophan hydroxylase in serotonin producing cells: demonstration of ATP-dependent proteolytic degradation. *FEBS Lett.* **368,** 151–154.
41. Kojima M., Oguro K., Sawabe K., et al. (2000) Rapid turnover of tryptophan hydroxylase is driven by proteasomes in RBL2H3 cells, a serotonin producing mast cell line. *J. Biochem. (Tokyo)* **127,** 121–127.
42. Iida Y., Sawabe K., Kojima M., Oguro K., Nakanishi N., and Hasegawa H. (2002) Proteasome-driven turnover of tryptophan hydroxylase is triggered by phosphorylation in RBL2H3 cells, a serotonin producing mast cell line. *Eur. J. Biochem*. **269,** 4780–4788.
43. McKinney J., Knappskog P. M., and Haavik J. (2005) Different properties of the central and peripheral forms of human tryptophan hydroxylase. *J. Neurochem.* **92,** 311–320.
44. Abbar M., Courtet P., Bellivier F., et al. (2001). Suicide attempts and the tryptophan hydroxylase gene. *Mol. Psychiatry* **6,** 268–273.
45. Rujescu D., Giegling I., Sato T., Hartmann A. M., and Moller H. J. (2003) Genetic variations in tryptophan hydroxylase in suicidal behavior: analysis and meta-analysis. *Biol. Psychiatry* **54,** 465–473.
46. Bellivier F., Leboyer M., Courtet P., et al. (1998) Association between the tryptophan hydroxylase gene and manic-depressive illness. *Arch. Gen. Psychiatry* **55,** 33–37.
47. Furlong R. A., Ho L., Rubinsztein J. S., Walsh C., Paykel E. S., and Rubinsztein D. C. (1998) No association of the tryptophan hydroxylase gene with bipolar affective disorder, unipolar affective disorder, or suicidal behaviour in major affective disorder. *Am. J. Med. Genet.* **81,** 245–247.
48. Kirov G., Owen M. J., Jones I., McCandless F., and Craddock N. (1999) Tryptophan hydroxylase gene and manic-depressive illness. *Arch. Gen. Psychiatry* **56,** 98,99.
49. McQuillin A., Lawrence J., Kalsi G., Chen A., Gurling H., and Curtis D. (1999) No allelic association between bipolar affective disorder and the tryptophan hydroxylase gene. *Arch. Gen. Psychiatry* **56,** 99–101
50. Tsai S. J., Hong C. J., and Wang Y. C. (1999) Tryptophan hydroxylase gene polymorphism (A218C) and suicidal behaviors. *Neuroreport* **10,** 3773–3775.
51. Courtet P., Jollant F., Castelnau D., Buresi C., and Malafosse A. (2005) Suicidal behavior: relationship between phenotype and serotonergic genotype. *Am. J. Med. Genet. C Semin. Med. Genet.* **133,** 25–33.
52. Cervo L., Canetta A., Calcagno E., et al. (2005) Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. *J. Neurosci.* **25,** 8165–8172.
53. Crowley J. J., Blendy J. A., and Lucki I. (2005) Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology (Berl)* **183,** 257–264.
54. Lipton S. A. and Kater S. B. (1989) Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. *Trends Neurosci.* **12,** 265–270.
55. Lauder J. M. (1993) Neurotransmitters as growth regulatory signals: role of receptors and second messengers. *Trends Neurosci.* **16,** 233–239.
56. Levitt P., Harvey J. A., Friedman E., Simansky K., and Murphy E. H. (1997) New evidence for neurotransmitter influences on brain development. *Trends Neurosci.* **20,** 269–274.

57. Azmitia E. C. (2001) Modern view on an ancient chemical: serotonin effects on proliferation, maturation, and apoptosis. *Brain Res. Bull.* **56,** 414–424.
58. Vitalis T. and Parnavelas J. (2003) Serotonin and cortical development. *Exp. Neurol.* **25,** 245–256.
59. Hendricks T. J., Francis N., Fyodorov D. J. and Deneris E. S. (1999) The ETS domain factor Pet-1 is an early and precise marker of central 5-HT neurons and interacts with a conserved element in serotonergic genes. *J. Neurosci.* **19,** 10,348–10,356.
60. Pfaar H., von Holst A., Vogt Weisenhorn D. M., Brodski C., Guimera J., and Wurst W. (2002) mPet-1, a mouse ETS-domain transcription factor, is expressed in central serotonergic neurons. *Dev. Genes Evol.* **212,** 43–46.
61. De Vitry F., Hamon M., Catelon J., Dubois M., and Thibault J. (1986) Serotonin initiates and autoamplifies its own synthesis during mouse central nervous system development. *Proc. Natl Acad. Sci. USA* **83,** 8629–8633.
62. Galter D. and Unsicker K. (2000) Sequential activation of the 5-HT1A serotonin receptor and TrkB induces the serotonergic neuronal phenotype. *Am. J. Anat.* **15,** 446–455.
63. Whitaker-Azmitia P. M. and Azmitia E. C. (1989) Stimulation of astroglial serotonin receptors produces culture media which regulates growth of serotonergic neurons. *Brain Res.* **497,** 80–85.
64. Salichon N., Gaspar P., Upton A. L., et al. (2001) Excessive activation of serotonin (5-HT) 1B receptors disrupts the formation of sensory maps in monoamine oxidase and 5-HT transporter knock-out mice. *J. Neurosci.* **21,** 884–896.
65. Luna B. and Sweeney J. A. (2001) Studies of brain and cognitive maturation through childhood and adolescence: a strategy for testing neurodevelopmental hypotheses. *Schizophr. Bull.* **27,** 443–455.