doi:10.1016/j.jmb.2006.08.051 J. Mol. Biol. (2006) 363, 345–354

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Requirement of Tryptophan Hydroxylase During Development for Maturation of Sensorimotor Gating

Kazuhiro Nakamura[1]*, Yoshimasa Koyama[2], Kazumi Takahashi[2] Hiromichi Tsurui[1], Yan Xiu[1], Mareki Ohtsuji[1], Qing Shun Lin[1] Yuko Sugawara[3], Keiko Sawabe[3], Akiko Ohashi[3], Tohru Ohnuma[4] Heii Arai[4], Hiroyuki Nishimura[5], Hiroyuki Hasegawa[3] and Sachiko Hirose[1]

[1]*Department of Pathology Juntendo University School of Medicine, Tokyo 113-8421 Japan*

[2]*Department of Physiology Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan*

[3]*Department of Bioscience Teikyo University of Science and Technology, Uenohara Yamanashi 409-0193, Japan*

[4]*Department of Psychiatry Juntendo University School of Medicine, Tokyo 113-8421 Japan*

[5]*Department of Biomedical Engineering, Toin Human Science and Technology Center Toin University of Yokohama Yokohama 225-8502, Japan*

**Corresponding author*

Deficits in sensorimotor gating, a function to focus on the most salient stimulus, could lead to a breakdown of cognitive integrity, and could reflect the "flooding" by sensory overload and cognitive fragmentation seen in schizophrenia. Sensorimotor gating emerges at infancy, and matures during childhood. The mechanisms that underlie its development are largely unclear. Here, we screened the mouse genome, and found that tryptophan hydroxylase (TPH) is implicated in the maturation of sensorimotor gating. TPH, an enzyme involved in the biosynthesis of serotonin, proved to be required only during the weaning period for maturation of sensorimotor gating, but was dispensable for its emergence. Proper serotonin levels during development underlie the mature functional architecture for sensorimotor gating *via* appropriate actin polymerization. Thus, maintaining proper serotonin levels during childhood may be important for mature sensorimotor gating in adulthood.

Keywords: actin; 5-HT; psychiatric disorder

Introduction

Attention and information processing dysfunctions have long been considered important in understanding psychiatric disorders.[1] Sensorimotor gating is a fundamental brain function, by which excess or trivial stimuli are screened or "gated out" of awareness by central inhibitory mechanisms in the early stage of information processing.[2] Sensorimotor gating is theoretically and reliably measured by the degree of prepulse inhibition (PPI), the suppression of the startle response by a relatively weak preceded stimulus[2,3] first documented in animals in the mid 1860s. In addition to its cognitive significance, sensorimotor gating has also psychology's increasing interest. Reduced PPI is shared by

Present address: Y. Koyama, Department of Science and Technology, Fukushima University, Fukushima 960-1296, Japan.

Abbreviations used: 5-HT, 5-hydroxytryptamine (serotonin); 5-HTP, 5-hydroxytryptophan; NZB, New Zealand Black; NZW, New Zealand White; PCPA, *p*-chlorophenylalanine; TPH, tryptophan hydroxylase; PPI, prepulse inhibition; QTL, quantitative trait loci; LOD, logarithm of the odds.

E-mail address of the corresponding author: kaz@med.juntendo.ac.jp

0022-2836/$ - see front matter

several common psychiatric and neurodegenerative disorders such as schizophrenia, Huntington's disease, Tourette's syndrome, schizotypal disorder, obsessive-compulsive disorder, and attention-deficit hyperactivity disorder.[4] Based on the gating deficit theory that schizophrenic patients should have increased distractibility due to their impaired ability to screen out irrelevant cues, gating deficits could lead to a breakdown of cognitive integrity and difficulty in distinguishing self from nonself[5,6] and could reflect the "flooding" by sensory overload and cognitive fragmentation seen in schizophrenia.[1] Indeed, antipsychotic agents used in the treatment of schizophrenia can increase PPI and reverse drug-induced deficits in animal models of sensorimotor gating.[7] Furthermore, some studies have shown that deficits in sensorimotor gating correlate with negative symptoms and thought disorder in schizophrenic patients.[8–10] Meincke *et al.* suggested that PPI deficits in schizophrenic patients appeared to be state-dependent.[11] Relative to control subjects, patients with schizophrenia had lower PPI only in the acute, but not in the improved clinical state. Larger PPI deficits were associated with more severe formal thought disorder and bizarre behavior.

An extensive series of anatomical and pharmacological approaches characterizing PPI have elicited the regions responsible, and transmitters involved in adulthood.[12,13] Studies involving rats indicate that multiple transmitter systems modulate PPI including dopamine, glutamate, acetylcholine, GABA, and serotonin (5-hydroxytryptamine (5-HT)). In regard to serotonergic influences on PPI, agonists and antagonists of 5-HT receptors including 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors modulate PPI in the adult.[14] The functional organization of the ultimate wiring patterns of the adult brain can be made up in the course of development through brain maturation. Subtle PPI can be obtained in infants. Then, a progressive increase in PPI occurs by eight years of age.[15–17] It is, therefore, likely that the formation of mature neural circuits for sensorimotor gating is achieved during childhood. The 5-HT system is an attractive candidate that underlies the maturation of sensorimotor gating during childhood because 5-HT is essential for the development of 5-HT neurons in an autocrine manner[18] in addition to its action as a crucial neurotransmitter in various regions in the adult brain. Changes in brain 5-HT content during development could disturb the development of 5-HT neurons involved in the PPI circuit, which might impair adult PPI. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of 5-HT. There are two isoforms (TPH1 and TPH2) of TPH. The distinction between the two was clearly demonstrated by Walther and colleagues who found the newer isoform, TPH-2.[19] They found part of a *TPH* gene on mouse chromosome 12 in the Genbank database. Subsequently, they identified homologous genes in rats and humans. Then, the tryptophan-hydroxylating activity of TPH2 was demonstrated.[19,20] *TPH2* mRNA is preferentially expressed in the brain.[19] In contrast, *TPH1* mRNA 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.[21]

Previous studies using recombinant congenic strains have demonstrated wide variation in PPI,[22,23] indicating that PPI is under genetic control. However, genes regulating the development of PPI have not yet been identified using genome screening. Considering neurodevelopmental models of psychiatric disorders,[24] the identification of genes involved in the development of PPI may be relevant to the development of gate-intrusive psychiatric disorders.

Here, genome-wide screening using available mouse microsatellite markers and backcrossed mice were used to determine the molecule relevant to the development of sensorimotor gating, which affects the function in adults. We found that TPH, a rate-limiting enzyme in 5-HT biosynthesis, is required for the maturation of sensorimotor gating during development. Further analyses with pharmacological and cell biological approaches revealed that reduced 5-HT levels during development result in an enhanced actin polymerization of 5-HT neurons.

Results

TPH1 gene is implicated in the development of sensorimotor gating

We found that the New Zealand White (NZW) mouse is a strain exhibiting a low PPI level, and compared the developmental time-course of PPI in NZW mice with that in New Zealand Black (NZB) mice. As shown in Figure 1(a), when PPI emerged at P14, the levels were comparable between the two strains. A robust increase was observed between P14 and P24, during which time a difference between the two strains became apparent. The difference observed at P24 essentially persisted until adulthood. Statistical analyses revealed a significant effect of strain (ANOVA, $F_{1,\ 81}=13.4$, $P<0.001$). An effect of age was also observed ($F_{4,\ 81}=42.8$, $P<0.001$), and there was interaction between age and strain ($F_{4,\ 81}=3.0$, $P=0.02$). Post hoc tests demonstrated a significant difference between NZB and NZW at P24 ($P=0.008$), P35 ($P<0.001$), and P65 ($P=0.046$). A significant increase was found between P14 and P24 in NZB mice ($P<0.001$), but not in NZW mice ($P=0.09$). The prominent difference found during the developmental stage allowed us to screen for the system critical for the maturation of PPI by comparing the NZW genome with the NZB genome. Since the PPI value was significantly higher in NZB/NZW F1 (B/W F1) mice than NZW mice at two months of age (Figure 1(b)), we performed a quantitative trait loci (QTL) genome-wide scan of PPI using B/W F1 × NZW backcrossed mice at two months old.