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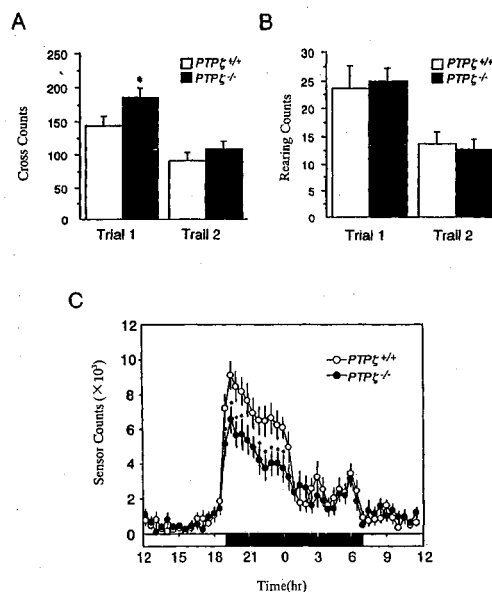
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(54) **METHOD OF SCREENING PTP ZETA ACTIVITY PROMOTER OR INHIBITOR**

(57) An object of the present invention is to provide a remedy for dysfunction of central monoamine pathway, a method for screening a PTP ζ inhibitor or activator, which is useful as a remedy for gastric ulcer caused by *Helicobacter pylori* or pleiotrophin which is a heparin-binding secretory protein, and a non-human model animal being hyposensitive to a stimulant drug, VacA which is a toxin of *Helicobacter pylori*, or pleiotrophin by utilizing the physiological function of PTP ζ . After administering a subject material to PTP ζ knockout mice and wild-type mice, PTP ζ activity in the PTP ζ knockout mice and the wild-type mice is compared and evaluated to screen a PTP ζ inhibitor or activator. Examples of the comparison and the evaluation of the PTP ζ activity include the comparison and the evaluation of the function of central monoamine pathway such as changes in the level of central monoamine metabolism, sensitivity to a stimulant drug, the presence of dysfunction of mesolimbic dopamine pathway, level of acclimation to new circumstances, or stress-responsiveness, and the comparison and the evaluation of the level of binding to VacA, a toxin of *Helicobacter pylori*, or pleiotrophin.

FIG. 1



Description**Technical Field**

5 **[0001]** The present invention relates to a remedy for dysfunction of central monoamine pathway with the use of a non-human animal such as a mouse or the like which is generated by a homologous recombination technique for genes and is deficient in its receptor-type protein tyrosine phosphatase (PTP ζ /RPTP β) gene, a screening of a remedy for gastric ulcer caused by *Helicobacter pylori* or the like, a non-human model animal being hyposensitive to a central stimulant drug (an addictive drug) and a non-human model animal being hyposensitive to VacA, a toxin of *Helicobacter pylori*, or the like.

Background Art

15 **[0002]** When a cell receives a stimulus from outside, its intracellular signaling pathway is activated to induce proliferation, differentiation, apoptosis and the like of the cell. Tyrosine phosphorylation of intracellular proteins acts an extremely important role in various phases of the signaling pathway, and the state of tyrosine phosphorylation of each protein is always regulated by dynamic equilibrium of delicate balance of two families of enzymes, tyrosine kinase (PTK) and tyrosine phosphatase (PTP). It is known that this tyrosine phosphorylation of proteins is involved in controlling the efficiency of neural circuit formation and neurotransmission in brains (SEITAI NO KAGAKU Vol. 48, No. 6, 534-538, (1997); PROTEIN, NUCLEIC ACID AND ENZYME Vol. 43, No. 8, 1136-1143 (1998)), and is important for the formation and the maintenance of the functions in an immune system and other organs (PROTEIN, NUCLEIC ACID AND ENZYME Vol. 43, No. 8, 1131-1135 (1998)). On the other hand, it is reported that the abnormal tyrosine phosphorylation of proteins is involved in defects in neural circuit formation, disturbance of memory and learning, abnormal apoptosis, tumorigenesis or the like (PROTEIN, NUCLEIC ACID AND ENZYME Vol. 43, No. 8, 1186-1192 (1998)).

25 **[0003]** To date, more than 80 kinds of PTP have been identified, and it is presumed that the number of genes of PTP in human would reach to 500. Similar to PTK, PTP is classified into two types: a receptor type and a non-receptor type. A receptor-type PTP has two or one enzymic domain intracellularly, and is classified into several groups according to the characteristics of its extracellular domain. PTP ζ , which has a carbonic anhydrase domain in N-terminal, has been identified as a receptor-type tyrosine phosphatase specific to the central nervous system. The inventors of the present invention have reported that PTP ζ is a receptor of growth factors including pleiotrophin and midkine (J. Biol. Chem. 271, 21446-21452, 1996; J. Cell Biol. 142, 203-216, 1998; J. Biol. Chem. 274, 12471-12479, 1999). In addition, PTP ζ is known to interact with cell adhesion molecules which belong to the immunoglobulin super family, such as N-CAM, and is thought to be responsible for important functions in differentiation, migration and neurotransmission of neurons. The present inventors have already generated a PTP ζ gene-deficient mouse and reported that PTP ζ has expressed in both neurons and astrocytes (Neuroscience Letters 274, 135-138, 1998). The PTP ζ gene-deficient mouse has grown and propagated normally, and no major morphologic abnormality has been identified. However, the physiological role of PTP ζ has been hardly elucidated so far.

35 **[0004]** Recently, Hirayama has shown a possibility that PTP ζ functions as a receptor of VacA, an exotoxin secreted by *Helicobacter pylori* which is well known as a cause of gastric ulcer, through a test system using cell lines (J. Biol. Chem. 274, 36693-36699, 1999). VacA, a toxin of *Helicobacter pylori*, is detected in about 90% of patients who suffer from acute gastritis or gastric ulcer, and it is reported that a mouse orally administered with VacA, a toxin extracellularly secreted by *Helicobacter pylori*, shows the onset of acute gastritis.

45 **[0005]** The clarification of in vivo role of PTP ζ , whose physiological function has been conventionally unknown, makes it possible to provide findings and experimental materials that lead to the elucidation of onset mechanisms of diseases related to the physiological function of PTP ζ , and to the development of remedies of the diseases. An object of the present invention is to provide a remedy for dysfunction of central monoamine pathway, a method for screening a PTP ζ inhibitor or activator, which is useful as a remedy for gastric ulcer caused by *Helicobacter pylori* or the like, as a non-human model animal being hyposensitive to a central stimulant drug (an addictive drug) and as a non-human model animal being hyposensitive to VacA, a toxin of *Helicobacter pylori*, or the like, by utilizing the physiological function of PTP ζ identified with the use of a PTP ζ gene-deficient mouse.

50 **[0006]** As aforementioned, the present inventors have already generated the mouse with the use of a homologous recombination technique, but it was difficult to eliminate the influence of other genes from the mouse because the mouse was a hybrid wherein chromosomes of two lines of mouse, that is, 129/Sv and C57BL/6J, were mixed. Therefore, a pure line mouse was sufficiently backcrossed (4 generations) to be suitable for analyzing the physiological function of PTP ζ , and a PTP ζ gene-deficient mouse from which the influence of other genes was eliminated was generated. By comparative analysis of a mouse whose function of gene DNA that encodes the PTP ζ was deficient on its chromosome and a wild type mouse, it is revealed for the first time that the PTP ζ gene-deficient mouse, which has grown and propagated normally, and where no major morphologic abnormality has been identified, has dysfunction of central

monoamine pathway, such as changes in the level of central monoamine metabolism, hyposensitivity to a stimulant drug (methamphetamine), dysfunction of mesolimbic dopamine pathway, delay in acclimating to new circumstances and increase in stress-responsiveness. Further, it was found that PTP ζ , which is thought to be a receptor of VacA, a toxin of *Helicobacter pylori*, is expressed in gastric epithelial cell layer of a mouse. The present invention has thus been completed.

Disclosure of the Invention

[0007] The present invention relates to a method for screening a PTP ζ inhibitor or activator wherein a subject material is administered to a non-human animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and a wild-type non-human animal, and PTP ζ activities in these non-human animals are compared and evaluated (claim 1), the method for screening a PTP ζ inhibitor or activator according to claim 1, wherein the comparison and the evaluation of PTP ζ activity is the comparison and the evaluation of the function of central monoamine pathway (claim 2), the method for screening a PTP ζ inhibitor or activator according to claim 2, wherein the comparison and the evaluation of the function of central monoamine pathway is the comparison and the evaluation of changes in the level of central monoamine metabolism, sensitivity to a stimulant drug, the presence of dysfunction of mesolimbic dopamine pathway, level of acclimation to new circumstances, or stress-responsiveness (claim 3), the method for screening a PTP ζ inhibitor or activator according to claim 1, wherein the comparison and the evaluation of PTP ζ activity is the comparison and the evaluation of the level of binding to VacA, a toxin of *Helicobacter pylori* (claim 4), the method for screening a PTP ζ inhibitor or activator according to claim 1, wherein the comparison and the evaluation of PTP ζ activity is the comparison and the evaluation of the level of binding to pleiotrophin, a heparin-binding secretory protein (claim 5), the method for screening a PTP ζ inhibitor or activator according to any one of claims 1 to 5, wherein the non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome is purified by being backcrossed for 4 or more generations (claim 6), the method for screening a PTP ζ inhibitor or activator according to any one of claims 1 to 6, wherein the non-human animal is a mouse (claim 7), a PTP ζ inhibitor or activator obtained by the method for screening a proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor or activator according to any one of claims 1 to 7 (claim 8), the PTP ζ inhibitor or activator according to claim 8, wherein the PTP ζ inhibitor or activator is a binding inhibitor between PTP ζ and VacA, a toxin of *Helicobacter pylori* (claim 9), the PTP ζ inhibitor or activator according to claim 8, wherein the PTP ζ inhibitor or activator is a binding inhibitor between PTP ζ and pleiotrophin, a heparin-binding secretory protein (claim 10), a remedy for dysfunction of central monoamine pathway containing the proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor or activator according to claim 8 as an active component (claim 11), a remedy for gastric ulcer or gastritis caused by *Helicobacter pylori* containing the proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor according to claim 8 or 9 as an active component (claim 12), a remedy for gastric ulcer or gastritis caused by pleiotrophin containing the proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor according to claim 8 or 10 as an active component (claim 13).

[0008] The present invention also relates to a non-human model animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and which is hyposensitive to a central stimulant drug (an addictive drug) (claim 14), the non-human model animal according to claim 14, wherein the non-human animal is hyposensitive to a stimulant drug (claim 15), the non-human model animal according to claim 14 or 15, wherein the non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome is purified by being backcrossed for 4 or more generations (claim 16), the non-human model animal according to any one of claims 14 to 16, wherein the non-human animal is a mouse (claim 17), a non-human model animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and which is hyposensitive to VacA, a toxin of *Helicobacter pylori* (claim 18), a non-human model animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and which is hyposensitive to pleiotrophin, a heparin-binding secretory protein (claim 19), the non-human model animal according to claim 18 or 19, wherein the non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome is purified by being backcrossed for 4 or more generations (claim 20), and the non-human model animal according to any one of claims 18 to 20, wherein the non-human animal is a mouse (claim 21).

Brief Explanation of the Drawings

[0009]

Fig. 1 is a view showing the examination results of character phenotypes of PTP ζ -deficient mice in an open field test and circadian rhythm.

Fig. 2 is a view showing the changes of monoamine metabolism in the brains of PTP ζ -deficient mice.

Fig. 3 is a view showing the examination results of immunohistochemistry of dopamine pathway in PTP ζ -deficient mice.

Fig. 4 is a view showing the decrease of locomotor activity to methamphetamine and GBR 129909 in PTP ζ -deficient mice.

Fig. 5 is a view showing the abnormal DA neurotransmission in nucleus accumbens of PTP ζ -deficient mice.

Fig. 6 is a view showing the examination results of expression property of PTP ζ in dopamine pathway of adult mice brains.

Fig. 7 is a view showing the results of stress and fear behaviors in PTP ζ -deficient mice.

Fig. 8 is a view showing the results of exploration behavior of wild-type mice and PTP ζ -deficient mice to a novel object.

Fig. 9 is a view showing the examination results of PTP ζ expression in gastric epithelial cell layer of wild-type mice and PTP ζ -deficient mice.

Fig. 10 is a view showing the examination results of transcription (RT-PCR) and expression (Western blot) of PTP ζ in gastric epithelial cell layer of mice.

Fig. 11 is a view showing the results of gastric ulcer formation in wild-type mice and PTP ζ -deficient mice orally administered with VacA, a toxin of *Helicobacter pylori*.

Fig. 12 is a view showing the examination results of tyrosine phosphorylation of GIT1, a substrate molecule of PTP ζ , caused by stimulus with VacA.

Fig. 13 is a view showing the results of mucosal damage in gastric epithelium of mice caused by administration of pleiotrophin.

Best Mode for Carrying out the Invention

[0010] The method for screening a PTP ζ inhibitor or activator according to the present invention is not particularly limited as long as it is a method for screening wherein a subject material is administered to a non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome and a wild-type non-human animal, and PTP ζ activities in these non-human animals are compared and evaluated (analyzed). An example of the non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome includes a non-human animal wherein a part or the whole of its endogenous gene that encodes PTP ζ is inactivated by gene mutation such as disruption, deficiency, substitution or the like, and the function of expressing PTP ζ in wild type is lost. In addition, as the non-human animal according to the present invention, rodents including a mouse, a rat and a guinea pig are specifically exemplified, but not limited to these animals. The method for constructing a non-human animal whose function of gene that encodes PTP ζ is deficient on its chromosome is explained below with an example of a mouse whose function of PTP ζ gene is deficient on its chromosome.

[0011] A mouse whose function of PTP ζ gene is deficient on its chromosome, in other words, a PTP ζ knockout mouse can be constructed by the method described in the aforementioned paper written by the present inventors (Neuroscience Letters 274, 135-138, 1998), or other such methods. Specifically, the construction method comprises the steps of: a PTP ζ gene is screened by using a gene fragment obtained from mouse gene library through PCR or the like; a part or the whole of the screened PTP ζ gene is substituted with a marker gene, for example, a Lac-Z gene, a neomycin-resistant gene or the like, and if necessary, a gene such as a diphtheria toxin A fragment (DT-A) gene or a herpes simplex virus thymidine kinase (HSV-tk) gene is introduced into 5'-terminal side, to construct a targeting vector; this constructed targeting vector is linearized and introduced into an ES cell by microinjection, electroporation or other such methods and then homologously recombined; among the homologous recombinants, an ES cell resistant to X-gal staining or antibiotics such as G418, ganciclovir (GANC) or the like is selected. It is preferable to confirm whether the selected ES cell is the intended recombinant by Southern blotting or other such methods.

[0012] The above-mentioned recombined ES cell is microinjected into a mouse blastocyst, and then the blastocyst is transplanted into a recipient to generate a chimeric mouse. A heterozygous mouse can be obtained by intercrossing the chimeric mouse and a wild-type mouse, and a PTP ζ knockout mouse can be obtained by intercrossing the heterozygous mice. Further, by backcrossing the obtained knockout mouse with a pure line mouse, PTP ζ knockout mice whose genetic background other than PTP ζ is in uniform can be obtained. Any pure line mouse can be used as the pure line mouse, for instance, C57BL/6J is specifically exemplified. Examples of the method for confirming whether PTP ζ is present in the PTP ζ knockout mouse include; Southern blotting or the like of DNA separated from a part of a tail tip of a mouse obtained by the above-mentioned methods, Northern blotting or the like of RNA isolated from neurons or the like of the mouse, and Western blotting or the like of PTP ζ expression in the mouse.

[0013] As the method for screening a PTP ζ inhibitor or activator according to the present invention, a method wherein a subject material is respectively administered to a non-human animal whose function of gene that encodes PTP ζ is deficient on its chromosome, for example, a PTP ζ knockout mouse, and a wild-type mouse, preferably a wild-type

littermate, and PTP ζ activities in the PTP ζ knockout mouse and in the wild-type mouse are compared and evaluated is exemplified. Examples of the comparison and the evaluation of the PTP ζ activities mentioned above are, the comparison and the evaluation of the function of central monoamine pathway including the comparison and the evaluation of changes in the level of central monoamine metabolism, sensitivity to a stimulant drug, the presence of dysfunction of mesolimbic dopamine pathway, level of acclimation to new circumstances or stress-responsiveness; the comparison and the evaluation of the level of binding to VacA, a toxin of *Helicobacter pylori*, particularly the level of binding inhibition between PTP ζ and VacA, a toxin of *Helicobacter pylori*, and the comparison and the evaluation of the level of binding to pleiotrophin, a heparin-binding secretory protein, particularly the level of binding inhibition between PTP ζ and pleiotrophin, a heparin-binding secretory protein, that are explained in detail in the examples.

[0014] The method for administering a subject material in the method for screening a PTP ζ inhibitor or activator is not particularly limited, and oral or intravenous administration or the like can be used. In such method, after administering a subject material, PTP ζ activities in the PTP ζ knockout mouse and in the wild-type mouse are measured, and then compared and evaluated. The higher cerebral function in which monoamine pathway is involved varies widely, and it is known that its dysfunction and degeneration lead to various disease states. Consequently, in the case where dysfunction of central monoamine pathway in a PTP ζ knockout mouse is ameliorated/cured to the same level as the function of a wild-type mouse, the subject material is thought to be a PTP ζ activator such as a PTP ζ agonist, and the PTP ζ activator shows promise as a remedy for dysfunction of central monoamine pathway, for example, as a novel remedy not only for Parkinson's disease or Huntington's disease, which are serious neurodegenerative diseases, autism or the like, but also for nervous affections including manic-depression, attention-deficit disorder, and drug addiction. In addition, there is a possibility that the PTP ζ inhibitor discovered by the comparison and the evaluation of the function of central monoamine pathway is useful as a remedy for gastric ulcer and gastritis caused by VacA, a toxin of *Helicobacter pylori*, or pleiotrophin, a heparin-binding secretory protein mentioned below.

[0015] VacA, an exotoxin secreted by *Helicobacter pylori* which is well known as a cause of gastric ulcer, is detected in about 90% or more of the patients who suffer from acute gastritis or gastric ulcer, and it is known that a mouse orally administered with VacA, a toxin extracellularly secreted by *Helicobacter pylori*, shows the onset of acute gastritis. Consequently, in the case where the level of binding to VacA, a toxin of *Helicobacter pylori*, in a wild-type mouse is ameliorated to a same level as that in a PTP ζ knockout mouse, it means that the subject material is a PTP ζ inhibitor such as a PTP ζ antagonist and a binding inhibitor between PTP ζ and VacA, a toxin of *Helicobacter pylori*, and the PTP ζ inhibiting substance/PTP ζ inhibitor shows promise as a novel remedy for gastric ulcer and gastritis. There is a possibility that the above-mentioned PTP ζ activator discovered by the comparison and the evaluation of the level of binding to VacA, a toxin of *Helicobacter pylori*, is useful as a remedy for dysfunction of central monoamine pathway. The present inventors have revealed for the first time that pleiotrophin causes gastric ulcer by binding to PTP ζ , and the involvement of pleiotrophin in the formation of gastric ulcer has never been known. A PTP ζ -deficient mouse is a negative control of the formation of gastric ulcer caused by pleiotrophin.

[0016] Further, the present inventors have revealed for the first time that 18 kD pleiotrophin, a heparin-binding secretory protein, causes gastric ulcer by binding to PTP ζ . Therefore, in the case where the level of binding to pleiotrophin in a wild-type mouse is ameliorated to a same level as that in a PTP ζ knockout mouse, it means that the subject material is a PTP ζ inhibitor such as a PTP ζ antagonist and a binding inhibitor between PTP ζ and pleiotrophin, and the PTP ζ inhibiting substance/PTP ζ inhibitor shows promise as a novel remedy for gastric ulcer and gastritis. There is a possibility that the above-mentioned PTP ζ activator discovered by the comparison and the evaluation of the level of binding to pleiotrophin is useful as a remedy for dysfunction of central monoamine pathway.

[0017] The non-human model animal hyposensitive to a stimulant drug (an addictive drug) according to the present invention is not particularly limited as long as it is a PTP ζ knockout mouse whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome, or the like, but a knockout backcrossed for 4 or more generations is preferable. The usefulness of a PTP ζ knockout mouse, in particular, its hyposensitivity to a stimulant drug (an addictive drug) had not been known until it was revealed for the first time by the present inventors. The term "hyposensitivity" here means lower level of reactivity to a stimulant drug in comparison to the reactivity level of wild-type non-human animals. The non-human model animal hyposensitive to a stimulant drug (an addictive drug) according to the present invention includes those from slightly hyposensitive ones to totally unresponsive ones. In addition, as the stimulant drug (the addictive drug), methamphetamine, amphetamine that has a same effect as methamphetamine, cocaine and cannabis that have a same effect as dopamine transporter inhibitor GBR 12909, LSD that has a same effect as p-chloro-amphetamine, which is a serotonin liberator, selective serotonin reuptake inhibitor (SSRI) used as an antidepressant such as fluoxetine, fluvoxamine, paroxetine, sertraline or the like, are exemplified. The non-human model animal hyposensitive to a central stimulant drug (an addictive drug) according to the present invention is hyposensitive to a stimulant drug (an addictive drug), and in addition, shows abnormal neurological properties including the changes in the level of central monoamine metabolism, dysfunction of mesolimbic dopamine pathway, delay in acclimating to new circumstances or increase in stress-responsiveness, as described in detail in examples mentioned below. Consequently, the non-human model animal is not only useful as an

experimental model animal for elucidating a signal transmission mechanism in the central nervous system such as a mechanism of response to a central stimulant drug (an addictive drug), but also available for screenings of novel remedies for drug addiction or nervous affections as mentioned above.

5 **[0018]** The non-human model animal hyposensitive to VacA which is a toxin of *Helicobacter pylori* or pleiotrophin which is a heparin-binding secretory protein according to the present invention is not particularly limited as long as it is a PTP ζ knockout mouse whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome, or the like, but a knockout mouse backcrossed for 4 or more generations is preferable. The usefulness of a PTP ζ knockout mouse, in particular, its hyposensitivity to VacA which is a toxin of *Helicobacter pylori* or pleiotrophin which is a heparin-binding secretory protein had not been known until 10 it was revealed by the present invention. The non-human model animal hyposensitive to VacA which is a toxin of *Helicobacter pylori* or pleiotrophin which is a heparin-binding secretory protein according to the present invention is not only useful as an experimental model animal for elucidating the onset mechanism of gastric ulcer caused by VacA which is a toxin of *Helicobacter pylori* or pleiotrophin which is a heparin-binding secretory protein, but also available as an advantageous negative control for the screening of a novel remedy for gastric ulcer as mentioned above.

15 **[0019]** The present invention is explained below more specifically with reference to examples, however, the present invention is not limited to these examples.

Example 1 (Physiological role of PTP ζ in central dopamine pathway and monoamine pathway, and usefulness of a PTP ζ -deficient mouse)

20 **[0020]** Though large amount of PTP ζ is expressed in the central nervous system, its neurophysiologic role has been unknown. Therefore, the importance of PTP ζ molecule in higher cerebral function was examined at individual level by comparing and analyzing a PTP ζ gene-deficient mouse and a wild-type mouse behaviorally and neuropharmacologically.

25 Example 1-1 [Method]

(A-1 Animals)

30 **[0021]** A PTP ζ -deficient mouse was generated by the method described in the aforementioned paper written by the present inventors (Neuroscience Letters 274, 135-138, 1998). A LacZ gene was inserted into the position immediately after the translation initiation codon in exon 1 of a PTP ζ gene of a mutant mouse and accordingly, a LacZ gene was expressed under the control of expression regulatory unit of the PTP ζ gene. In this experiment, a knockout mouse was backcrossed with inbred C57BL/6J line for 4 generations, and male littermates of 2 to 5 months old were used. The 35 animals were housed in an animal care facility at 25.degrees C., with a 12/12 hours light-dark cycle and fed food and water ad libitum. Animals were cared in accordance with the institutional guidelines.

(A-2 Behavioral experiment)

40 **[0022]** A behavioral experiment was conducted during the light cycle from 7:00 a.m. to 7:00 p.m.

A-2-1

45 **[0023]** In an open field test, a mouse was placed in the center of a gray round field (internal diameter: 80 cm, height: 40 cm) divided into 25 equal segments by grids. Locomotor activity was measured as the number of line crossings of the grid during 5 minutes.

A-2-2

50 **[0024]** Circadian motility was measured with an infrared ray passive sensor system (AB system 24A, Neuroscience, Inc.). For the measurement, a sensor was set on the top of a normal mouse cage (30×20×13 cm) where the animals were housed individually with ad libitum food and water for 7 days. Analysis and evaluation were made with the measured data of the last 3 days.

55 A-2-3

[0025] In a forced swimming test, a mouse was forced to swim for 15 minutes in 2 consecutive days in a cylindrical container (diameter: 8 cm, height: 20 cm) which contained water at 22.degree. C. to a depth of 8 cm. Locomotion was

measured every 5 minutes with an infrared monitoring apparatus (SCANET MV-10, Toyo Sangyo Co. Ltd.).

A-2-4

5 **[0026]** An elevated plus maze consisted of a central area (6×6 cm) and 4 arms placed 50 cm above floor. Two "closed arms" were enclosed within walls (30 cm), and the other two "open arms" had low rims (1 cm). A mouse was placed in the center of the maze and the number of entries and time spent in the open and closed arms were counted for 5 minutes under a dim light condition (about 80 lux).

10 A-2-5

[0027] An exploration behavior to a novel object was evaluated with an open field container and a plastic cube (9 cm on a side). On the day before the experiment, a mouse was placed in an open field for about 10 minutes to be acclimated to the circumstance in advance. On the day of the experiment, the mouse was placed in the field that contained nothing, and observed for 9 minutes. Then, a cube-shaped plastic block was placed quietly in the center of the central area of the field, and the mouse was further observed for 9 minutes. As to the result of the observation, the number of times that the mouse crossed the partition lines in the open field was counted and evaluated as mobility, and the number of times that the mouse crossed the partition lines in the whole area of the field and a certain area in the center of the field were evaluated respectively.

20

(A-3 Pharmacological experiments)

[0028] The effects of psychostimulant drugs on locomotor activity were measured in a clear acrylic chamber (40×40×40 cm) by measuring animal movements with an electromagnetic activity monitoring system (sensor unit 0603, scanner 1099, Panlab). Before drug administration, the animals were acclimated to the measurement circumstance for at least 90 minutes. The animals were then administered with a stimulant drug, methamphetamine (METH) (1 mg/kg, s.c.), GBR12909 (2 mg/kg, i.p.), apomorphine (1 mg/kg, s.c.), or p-chloro-amphetamine (2 mg/kg, i.p.), and saline as administered to a control, and locomotor activity was monitored for the next 60 minutes.

30 (A-4-1 Quantification of dopamine and its metabolites)

[0029] Brain tissues were dissected on ice and extracted by sonication in a solution containing 10 mM HClO₄, 0.1 mM sodium pyrosulfite, 20 μM EDTA-2Na and 10 pg/ml (±) isoproterenol as an internal standard, and the extract was centrifuged at 15,000 rpm for 10 minutes. Dopamine and its metabolites in the supernatant were measured by auto-
35 mated HPLC with an electrochemical detector (Coulchem II, MC Medical, Inc.) under the following condition. The separation was performed on MCM C-18 column (4.6×150 mm, MC Medical, Inc.) using 50 mM acetate-citrate buffer (pH 3.0) containing 3.1% acetonitrile, 7.6% methanol, 4.4 mM sodium 1-heptanesulfonate, and 0.1 mM EDTA-2Na, at a flow rate of 1.0 ml/min in a column-chamber maintained at 37.degree. C. The working electrode was set at +450 mV.

40 (A-4-2 In vivo microdialysis)

[0030] Mice were anaesthetized with sodium pentobarbital (80 mg/kg i.p.) and placed in a stereotaxic apparatus. A hole was drilled through the skull and an intracerebral guide cannula (CMA 11, CMA/Microdialysis AB) was inserted into the nucleus accumbens (NAC) and fixed with dental cement. The stereotaxic coordinates for implantation of the
45 guide cannula in both wild-type and PTPζ-deficient mice were: anterior, +1.1 mm, ventral, +3.6 mm, lateral, +1.0 mm to the surface of bregma. 24 hours after the surgery, mice were chained to a counterbalance lever arm attached to a locomotor activity chamber (30x30 cm) with an infrared monitoring apparatus (SCANET LC-10, Toyo Sangyo Co., Ltd.). Dialysis probes (membrane length 1 mm; external diameter 0.24 mm; cuprophane CUP 11, CMA/Microdialysis AB) were inserted through the guide cannula and perfusion was performed with artificial cerebrospinal fluid (ACSF), pH
50 7.4, (in mM; 145.0NaCl, 2.7 KCl, 1.2 CaCl₂, 1.0 MgCl₂, and 2.0 NaH₂PO₄) at a flow rate of 2 μl/min. Perfusates were collected from freely moving animals every 20 minutes in a tube containing 20 μl of 0.4 M HClO₄ (CMA 170). Samples were collected and assayed using HPLC-ECD as described above except for the following modifications: the column was MCM C-18 (4.6×150 mm) and the buffer contained 75 mM NaH₂PO₄, 1.7 mM 1-octanesulfonic acid sodium salt, 0.1 mM triethylamine, 25 μM EDTA-2Na and 10% acetonitrile, at flow rate of 0.55 ml/min at 30.degree. C. The electrode
55 was set at +320 mV. METH administration (1 mg/kg s.c.) or local infusion of high K⁺ solution (in mM: 47.7 NaCl, 100.0 KCl, 1.2 CaCl₂, 1.0 MgCl₂, and 2.0 NaH₂PO₄, pH 7.4) through the microdialysis probe was performed after the state became steady.

(A-5 Immunohistochemistry)

[0031] Mice were anaesthetized with sodium pentobarbital and perfused and fixed with 4% paraformaldehyde solution. Brains were dissected and incubated overnight in 0.1 M phosphate buffer (PB) containing 30% sucrose at 4.degree. C. The brains were cut into 40 μm sections on a cryostat. The sections were treated with 20 mM PB-saline (PBS) containing 3% H_2O_2 and 0.05% NP-40 for 30 minutes, and then blocked with 10% normal goat serum and 0.05% TritonX-100 in PBS. The sections were then incubated overnight with rabbit anti-tyrosine hydroxylase (1:1000) (AB152, Chemicon International Inc.). The binding of specific antibodies was detected by using an ABC peroxidase kit (Vectors Laboratories, Inc.) according to the manufacture's instructions with 3,3-diaminobenzidine (DAB) and H_2O_2 as the substrate. To detect the β -galactosidase which reflect the PTP ζ -gene expression, tissue sections from the heterozygous mutant mice were subjected to X-gal staining before immunohistochemical analyses (Neuroscience Letters 274, 135-138, 1998).

(A-6 Dopamine uptake assay)

[0032] Dopamine (DA) uptake was measured as follows. Striatal synaptosome tissue pools (3 animals/pool) were homogenized in 100 volume (v/w) of 0.32 M sucrose in a teflonglass homogenizer and centrifuged at 12,000 rpm for 10 minutes. The crude synaptosomal pellet was resuspended in 0.32 M sucrose at a concentration of 30 mg/ml (original wet weight) and divided into 30 μl aliquots. Each aliquot was added with 270 μl of Krebs-Ringer phosphate buffer with increasing concentrations of [^3H] DA (final 1-10 nM, 88.5 Ci/nmol, Amersham Pharmacia Biotech UK Ltd.). After incubating the resultant solution for 3 minutes at 30.degree. C., uptake was quickly terminated by filtration through Whatman GF/C glass filters. The filters were rinsed three times with 2.5 ml ice-cold 0.32 M sucrose and measured by using a liquid scintillation counter at an efficiency of 50%. Blank values were measured in the presence of 10 μM GBR12909.

(A-7 Synthesis of cDNA from a single cell)

[0033] The cDNA preparation from single cells was carried out as described previously (J. Neuroscience 18, 3124-3137, 1998). Mice brains were removed quickly after decapitation, and cut into 400 μm thick coronal midbrain slices with a microslicer while bathed in low Ca^{2+} HEPES-buffered saline (in mM: 140.0 Na isethionate, 2.0 KCl, 4.0 MgCl_2 , 0.1 CaCl_2 , 23.0 glucose, and 15.0 HEPES). The slices were maintained for one hour at room temperature in NaHCO_3 buffered saline (having 126.0 NaCl, 2.5 KCl, 2.0 MgCl_2 , 26.0 NaHCO_3 , 1.25 NaH_2PO_4 , 1.0 pyruvic acid, 0.2 ascorbic acid, 0.1 N^G -nitro-L-arginine, 1.0 kynurenic acid, and 10.0 glucose, adjusted to be pH 7.4 with NaOH, in mM). The ventral tegmental area and substantia nigra were cut out and put in low Ca^{2+} , and then treated with 1 mg/ml pronase in HEPES-buffered HBSS at 35.degree. C. for 30 minutes in an oxygenated Cell-stir chamber (Wheaton, Inc.). The tissue was rinsed three times with low Ca^{2+} HEPES-buffered saline and its cells were dissociated by repeated pipetting with Pasteur pipettes. The cell suspension was poured into a 35 mm Lux Petri dish containing HEPES-buffered HBSS, which was mounted on the stage of a microscope. After allowing cells to settle down, the solution was replaced by HEPES buffer. The content of the cells were aspirated into an electrode pipette filled with diethyl pyrocarbonate (DEPC)-treated water. The content was immediately recovered and added to a mixture of 5 μl DEPC-treated water (H_2O), 0.5 μl RNAsin (28,000 U/ml, Promega Corporation), 0.5 μl dithiothreitol (DTT, 0.1 M), and 0.1 μl random hexamer primer (50 ng/ μl). The resulting mixture was heated at 70.degree. C. for 10 minutes, and then iced quickly for 5 minutes. The cDNA was synthesized by adding 1 μl of Superscript II reverse transcriptase (200 U/ μl) (Life Technologies), 4 μl of 5 \times reaction buffer, and 1 μl of 10mM dNTP to cell mRNA. After conducting the reaction in a total volume of 20 μl at 42.degree. C. for 50 minutes, a treatment was performed at 70.degree. C. for 15 minutes.

(A-8 Multiplex and nested PCR)

[0034] Multiplex and nested PCR were performed as previously described (EMBO J. 18, 833-846, 1999) with modifications. Single-cell cDNA was treated with RNase (1 μl , 2 U/ μl) at 37.degree. C. for 20 minutes. The primer sets of marker genes, tyrosine hydroxylase (TH, GenBank accession No. M69200), glutamic acid decarboxylase 67 (GAD67, accession No. Z49976), and glial acidic fiber protein (GFAP, accession No. K01347), were reported by Liss et al. (EMBO J. 18, 833-846, 1999), and the primer sets to amplify PTP ζ (accession No. U09357) were constructed in this experiment. The cDNA of TH, GAD67, GFAP, and PTP ζ were amplified simultaneously in a single tube by multiplex PCR using the first primer mixture (for PTP ζ from 5' to 3' ; sense [Seq. ID. No. 1]: GGT CCA CTG AAG TCC ACA GC; position 5512 to 5531, antisense [Seq. ID. No. 2]: TCT AGT ACA ATG TAT GTG CCC G; position 5948 to 5927). The initial multiplex-PCR was performed in a final volume of 20 μl containing 5 μl of the single-cell cDNA, 20 pmol of each primer, 200 μM dNTPs, 2 μl of 10 \times PCR buffer, and 1 U of EX-Taq (Takara Shuzo, Co., Ltd.). The PCR was conducted with a Thermal Cycler MP (Takara) under the following conditions: 94.degree. C. for 5 minutes followed by 25 cycles at 95.de-

gree. C. for 30 seconds, 62.degree. C. for 3 seconds, and 72.degree. C. for 2 minutes, with a treatment at 72.degree. C. for 5 minutes. The nested-PCR was carried out in four individual reactions in a final volume of 20 μ l containing 0.1 μ l of the initial PCR product, 200 μ M dNTPs, 2 μ l of 10 \times PCR buffer, 0.5 U of EX-Taq, and 10 pmol of each of primer pairs (for PTP ζ sense [Seq. ID. No. 3]: CGG GAG CTT CCT GGT CAA CCA G; position 5655 to 5677, antisense [Seq. ID. No. 4]: AGC ACG GGT AGG GAG TAC TC; position 5873 to 5824). To investigate the presence and the size of the amplified fragments, 5 μ l aliquots of the PCR products were separated by electrophoresis on 3% Nusiv 3:1 agarose gel (FMC BioProducts, Rockland), and visualized with ethidium bromide staining. The predicted sizes (bp) of the eight PCR-generated fragments were: 189 (PTP ζ), 377 (TH), 517 (GFAP), and 702 (GAD67). The primers for PTP ζ can only amplify the intracellular phosphatase domain D1 which is present only in the sequences of the PTP ζ -A and -B isoforms, but not in the PTP ζ -S which is also known as phosphacan (J. Biochem. (Tokyo), 123, 458-467, 1998).

Example 1-2 [Results]

(Behavioral phenotype of PTP ζ -deficient mice)

[0035] In order to elucidate the behavioral phenotype of PTP ζ -deficient mice, behavioral observation was conducted by an open field test (the above-mentioned methods A-1 and A-2-1). Locomotor activity (A) and rearing (B) of wild-type mice (PTP $\zeta^{+/+}$, n=10) and PTP ζ -deficient mice (PTP $\zeta^{-/-}$, n=12) were measured for consecutive 2 days by the open field test, and the results are shown in Figs. 1A and 1B, respectively. The data are presented as mean \pm SEM (*p<0.05). As a result, PTP ζ -deficient mice exhibited larger amount of locomotor activity than wild-type mice, and significantly high motility in the open field on the first day. However, 24 hours later, the change disappeared in the test (Fig. 1A). On the other hand, significant change was not found in values for rearing, an exploration behavior (Fig. 1B). It was revealed that the PTP ζ -deficient mice increased their responsibility of locomotor activity to novel circumstances.

[0036] Locomotor activity in home-cage condition was examined for consecutive days (the above-mentioned method A-2-2). Mice were housed individually for 7 days, and locomotor activity was analyzed with an infrared ray passive sensor system during the last 3 days (PTP $\zeta^{+/+}$, n= 14 and PTP $\zeta^{-/-}$, n=14). The results are shown in Fig. 1C. The data are presented as mean \pm SEM per each 30 minutes interval (*p <0.05). The PTP $\zeta^{-/-}$ mice showed a marked decrease in the peak of circadian activity during the early dark phase compared with the wild-type mice, and their activity peak immediately after lights were put off (19:00 to 24:00) was significantly low in comparison with the wild-type mice, though their activity rhythm was normal.

(Abnormal metabolism of monoamine in brain)

[0037] It has been known that locomotor activity and circadian rhythm are deeply linked to monoaminergic systems in the brain. Therefore, tissue contents of dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their metabolites (DOPAC, HVA, metabolites of DA; MHPG, a metabolite of NE; 5-HIAA, a metabolite of 5-HT) in the brains were measured by high-performance liquid chromatography (HPLC) and an electrochemical detector (ECD) (the above-mentioned method A-4-1). The results are shown in Fig. 2. The data are presented as mean \pm SEM from PTP $\zeta^{+/+}$ mice (n=7) and PTP $\zeta^{-/-}$ mice (n=7) (*p<0.05, **p<0.01). As a result, metabolism of monoamine in the brains of the PTP ζ -deficient mice was revealed to be apparently abnormal. There was almost no difference between the two genotypes in the tissue content of DA (A). However, the ratios of DOPAC/DA (B) and HVA/DA (C), 5-HIAA/5-HT (G) in several brain regions of the PTP ζ -deficient mice were markedly decreased compared to those of the wild-type mice. The content of NE in the PTP ζ -deficient mice increased compared to that of the wild-type mice.

(Immunohistological analysis of DA pathway)

[0038] Next, DA pathway of PTP ζ -deficient mice was analyzed immunohistologically for detecting the presence of histological change therein with a specific antibody to tyrosine hydroxylase, a marker enzyme of DA neurons (the method A-5). The results are shown in Fig. 3. Regions of wild-type mice (+/+, left side) and PTP ζ -deficient mice (-/-, right side) through the substantia nigra and ventral tegmental area (low-magnification photographs, a and b; and high-magnification photographs, c and d), and striatum (e and f) were analyzed immunohistochemically with polyclonal antibodies specific to tyrosine hydroxylase (TH). According to the results, no significant difference was identified between DA pathways of the PTP ζ -deficient mice and the wild-type mice, and DA pathway was considered to remain immunohistologically normal after PTP ζ became deficient. The scale bars, 500 μ m (a, b, e, and f), and 50 μ m (c and d).

(Decrease in sensitivity to stimulant drugs)

[0039] Methamphetamine (METH), a stimulant drug, acts on DA pathway and increases the locomotion of mice

markedly. In expectation of a possibility of functional change in DA pathway of PTP ζ -deficient mice, sensitivity to METH was analyzed by an electromagnetic locomotion measuring apparatus (the above-mentioned method A-3). The results are shown in Fig. 4. Mice were placed in an acrylic chamber, and locomotion was measured by the counts of electromagnetic sensors. PTP $\zeta^{-/-}$ mice (n=9) and PTP $\zeta^{+/+}$ mice (n=9) showed similar time course of locomotor activity before drug administrations. After an adequate acclimation (at least 90 minutes), methamphetamine (METH, 1 mg/kg, s. c.) or saline (arrow point) was injected, and it was indicated that the PTP $\zeta^{-/-}$ mice exhibited a significantly attenuated locomotor response compared to the wild-type mice (Fig. 4A). No change was observed in the control group administered with saline. The data are presented as mean \pm SEM per each 20 minutes interval ($p < 0.001$ for phenotype).

[0040] Effects of METH (1 mg/kg, s. c.), GBR12909 (2 mg/kg, i. p.), apomorphine (2 mg/kg, s. c.), p-chloro-amphetamine (2 mg/kg, i. p.) and saline on locomotor activity are presented in Fig. 4B as the total counts of locomotion during the 60 minutes interval after drug administration. Locomotor activity was measured in the same manner as above-mentioned. The data are presented as mean \pm SEM ($*p < 0.05$, $**p < 0.01$). PTP ζ -deficient mice showed similar hyposensitivity also to GBR12909, a specific inhibitor of DAT (Fig. 4B). On the other hand, though apomorphine, a non-specific agonist for DA receptors, shows similar effect by stimulating a postsynaptic neuron, responsiveness to apomorphine was normal (Fig. 4B). These results suggest that neurotransmitting function of a presynaptic neuron is abnormal in DA pathway of the PTP ζ -deficient mice. The PTP ζ -deficient mice were hyposensitive also to p-chloro-amphetamine that liberates 5-HT from a presynaptic region (Fig. 4B).

[0041] [^3H] DA was mixed with striatal synaptosomes and [^3H] DA uptake into tissues was measured (the above-mentioned method A-6-1). The homogenate of the striatal synaptosomes was incubated for 2 minutes at 30 degree C. in the presence of [^3H] DA (1 to 10 nM). The amount of DA uptake was analyzed by Eadie-Hofstee plot, and maximum velocity (V_{max} , pmol/2 min/mg protein) and affinity (K_m , nM) are presented in Table 1, as mean \pm SEM of three measurement values ($*P < 0.05$). From Table 1, it was found that both of maximum velocity (V_{max}) and affinity (K_m) of DA uptake amount are changed dynamically, though there was no difference in the number of DAT.

Table 1

Mice	^3H DA uptake	
	K_m (nM)	V_{max} (pmol/3 min/mg)
PTP $\zeta^{+/+}$	109.2 \pm 2.45	45.74 \pm 2.77
PTP $\zeta^{-/-}$	147.3 \pm 11.3	58.70 \pm 1.03

(Abnormality of DA neurotransmission in nucleus accumbens)

[0042] Effect of activating locomotor activity caused by administration of METH or GBR12909 is induced mainly by an increase of extracellular DA concentration in nucleus accumbens (NAC). The change of extracellular DA concentration in nucleus accumbens after the administration of METH was analyzed by microdialysis (the above-mentioned method A-4-2). METH-induced DA release in the NAC was analyzed by microdialysis, and the results are shown in Fig. 5A. Each value is mean \pm SBM from PTP $\zeta^{+/+}$ mice (n=7) and PTP $\zeta^{-/-}$ mice (n=6), and the percentage value to the average of 2 samples before METH stimulation. The PTP ζ -deficient mice exhibited a markedly diminished DA release evoked by administration of METH (1 mg/kg, s. c.) compared to the wild-type mice ($P < 0.001$ for phenotype). In the wild-type mice, the extracellular DA in nucleus accumbens definitely increased by the administration of METH (1 mg/kg, s. c.), on the contrary, in the PTP ζ -deficient mice, extracellular DA increasing response evoked by METH stimulation attenuated obviously in comparison to that of the wild-type mice (Fig. 5A).

[0043] It was confirmed that locomotor activity after METH administration attenuated in PTP ζ -deficient mice corresponding to the extracellular DA concentration. Locomotion of freely moving animals was analyzed by an infrared monitoring apparatus, confirming that PTP $\zeta^{-/-}$ mice exhibited a statistically attenuated METH-enhanced locomotion after METH stimulation compared to wild-type mice ($*p < 0.05$). The results are shown in Fig. 5B. In other words, it was identified that the cause of hyporesponsiveness to stimulant drugs was presented in the process of release and uptake of DA from a presynaptic region. On the other hand, when DA releases from synaptic vesicles were induced by causing depolarization by local administration of 100 mM KCl solution through microdialysis probe inserted into the nucleus accumbens, DA release response of PTP ζ -deficient mice was the same level as that of wild-type mice (Fig. 5C). In addition, as there was no change in the extracellular DA concentration in a steady state as well (Fig. 5D), it was revealed that PTP ζ was not essential to the release mechanism of DA synaptic vesicle.

(Role of PTP ζ in DA pathway)

[0044] It is very important to clarify the region of PTP ζ expression in elucidation of the role of PTP ζ in DA pathway.

As detailed expression pattern of PTP ζ in mature brain is unknown, the expression region of the molecule was identified from the expression pattern of a LacZ gene, a reporter gene of the PTP ζ gene, in this case. In other words, cells that were stained blue by X-gal were identified as PTP ζ -positive cells. Regional distribution of cells that display PTP ζ promoter activity in the DA circuit of adult mice was examined in adult heterozygous mice by X-gal staining (blue signal), and then by TH staining (brown signal), and the results are shown in Figs. 6A and B. A number of positive cells were observed in substantia nigra (SN) and ventral tegmental area (VTA), which are nuclei of origin of DA pathway, on the contrary, no cells were detected in striatum (ST) and nucleus accumbens (NAC), which are the main regions for receiving DA projections. This result is consistent with the result of pharmacological analysis wherein the functional change in the presynaptic region of DA pathway was identified by deficiency in PTP ζ .

[0045] Next, in order to confirm a possibility that PTP ζ functions directly in DA neurons, an analysis by RT-PCR using RNA prepared from single cells as a template, that is, single-cell RT-PCR, was conducted (the above-mentioned method A-7 and A-8). Single-cell RT-PCR using cDNA derived from mouse brain as a template was conducted, then the products were analyzed by agarose gel electrophoresis, followed by ethidium bromide staining, and the results are shown in the top of Fig. 6C. Phosphatase domain D1 of PTP ζ and PCR products of a marker gene, tyrosine hydroxylase (TH, dopamine neuron marker), glutamic acid decarboxylase (GAD67, GABA neuron marker) and glial fibrillary acidic protein (GFAP, astroglia cell marker) were detected in each position expected from its mRNA base sequence, that is, PTP ζ (189), TH (377), GAD67 (702), GFAP (517). The validity of these PCR products were verified by DNA sequencing after cloning. PCR amplification from genomic DNA did not occur because primers were set to sandwich an intron therebetween. In addition, PCR amplification was not observed in controls to which no reverse transcriptase was added. Representative examples being identified as TH-positive (DA neurons) and GAD-positive (GABA neurons) are shown at the bottom of Fig. 6C. As a result that 46 cells were collected from substantia nigra and ventral tegmental area of 4 mice and analyzed, 28 of TH-positive dopamine neurons and 6 of GAD67-positive GABA neurons were identified. Eight cells that amplified neither TH nor GAD, and 4 cells wherein amplification of GFAP was observed were excluded from the analysis. Amplification of PCR products of PTP ζ was observed in 83% of DA cells (Fig. 6D). In other words, it was proved that receptor-type PTP ζ having protein tyrosine dephosphorylating activity was expressed in most DA neurons in brains of adult mice.

(Change in responsiveness to forced swimming stress)

[0046] It is known that DA and 5-HT pathways are involved not only in locomotor activity but also in emotion and stress response. In a forced swimming stress test, mice were placed into a narrow pool under inescapable condition, and the swimming time was evaluated/analyzed in time course (the above-mentioned method A-2-3). It is presumed that though animals actively swim to escape from stress stimulus at first, they despair of inescapable situation and gradually become less mobile. A 15 minutes' test on the first day was conducted to induce a state of depression. In this test, there was no significant difference between the swimming time of deficient mice and wild-type mice (Fig. 7A). However, in a test conducted 24 hours later, it was shown that the PTP ζ -deficient mice swam longer than the wild-type mice (Fig. 7B). During the 15 minutes' test, mouse locomotion gradually decreased, and the activity level during the last 10 minutes was significantly greater in the PTP ζ -deficient mice (n=8) than in the wild-type controls (n=8). That is, it was found that the PTP ζ -deficient mice were hard to be acclimated to stress stimulus.

[0047] An elevated plus maze test, which is a general method for evaluating emotional behaviors, anxiety in particular, was conducted next (the aforementioned method A-2-4). In this test, mice are subjected to ambivalent condition wherein they try to avoid the open arms of maze because brightness makes them anxious, while they feel like exploring the site at the same time. In other words, animals that are susceptible to anxiety will avoid the open arms and spend longer time in the closed arms. On the other hand, mice having a strong desire to exploration will show more exploration behavior and in and out movement between the open and the closed arms. There was no apparent difference between PTP ζ -deficient mice (n=9) and wild-type controls (n=12) in their behaviors in the elevated plus maze (Fig. 7C). Further, as to the total number of entries into the arms of the maze, which is a measured value of exploration behavior, no difference was observed between the two phenotypes as well (Fig. 7D). The two kinds of mice were evaluated as comparable in these two factors, time spent in the open-the close arms (anxiety behavior) and the total number of entries into the arms (exploration behavior).

(Change in exploration behavior to a novel object)

[0048] Exploration behaviors to an object never seen before was examined (the above-mentioned method A-2-5). The results are shown in Fig. 8. Mobilities of PTP ζ -deficient mice (PTP ζ ^{-/-}, n=9) and wild-type controls (PTP ζ ^{+/+}, n=9) in the whole area of the open field (Fig. 8A) and the central area of the open field (Fig. 8B) are presented as mean \pm SEM per each 3 minutes (*p<0.05, **p<0.01). At the first measurement (Trial. 1), nothing was placed in the field during the first 9 minutes. It was found that both types of mice hardly stayed in the central area in this circumstance. Subsequently,

when a block (an object never seen before) was placed at the center of the field, mice showed exploration behavior to the block, and remarkably increased the frequency of going in and out of the central area. In accordance with this increase, overall mobility also increased. Further, the graph showed the time course decrease of exploration behaviors to the block. When comparing the two types of mice, PTP ζ ^{-/-} mice showed mobility twice or more higher than that of wild-type mice during the first 3 minutes in the central area where the block was placed, and kept showing significantly higher mobility until the first 6 minutes passed. 24 hours later, the second trial (Trial. 2) was conducted under the same condition. Mobility of wild-type mice (PTP ζ ^{+/+}) showed almost no increase, consequently, it was considered that the mice stopped exploring the block, on the other hand, PTP ζ ^{-/-} mice showed an increase in mobility which was considered to be caused by exploration behavior as before. When the third trial (Trial. 3) was conducted, significant difference between the two types of mice was no longer observed. These results indicate that PTP ζ ^{-/-} mice are slow in acclimating to a new circumstance.

Example 2 (Role of PTP ζ in gastric ulcer formation caused by VacA, a toxin of *Helicobacter pylori*)

[0049] The role of PTP ζ in gastric ulcer formation caused by *Helicobacter pylori*, in particular, immunohistological identification of PTP ζ in stomach, actual involvement of PTP ζ as a receptor of exotoxin VacA secreted by *Helicobacter pylori* in gastric ulcer formation, and the usefulness of a PTP ζ -deficient mouse as a negative control were considered.

Example 2-1 [Method]

(B-1 Immunohistochemical staining of PTP ζ in mouse stomachs)

[0050] Formalin-fixed and paraffin-embedded stomach tissue sections of wild-type and PTP ζ -deficient mice were immunostained with anti-6B4 proteoglycan polyclonal antibody that recognizes the extracellular region of PTP ζ . The tissue sections were deparaffinized in xylene, and dehydrated in ethanol. The sections were washed with phosphate buffered saline (PBS, pH 7.6), then subjected to microwave for 15 minutes in 10 mM citrate buffer (pH 6.0). The deparaffinized sections were incubated for 10 minutes with 0.3% hydrogen peroxide, and washed with PBS after blocking endogenous peroxidase. Subsequently, the sections were blocked with bovine serum albumin for 10 minutes, and washed with PBS. The treated tissue sections were incubated overnight with anti-6B4 proteoglycan polyclonal antibody (1:2000) in Tris-HCl buffer at 4.degree. C. containing a carrier protein and 15 mM sodium azide. After the sections were washed with PBS, their specific antibody binding was examined by DAKO Envision System (DAKO Corp.) by using DAB and H₂O₂ as substrates in accordance with manufacturer's protocol. The sections were slightly counter stained with hematoxylin.

(B-2 RT-PCR and Western blotting of PTP ζ in mouse stomachs)

[0051] RT-PCR of PTP ζ in mouse stomachs was conducted as follows. TRIzol Reagent (Invitrogen) was used for extracting RNA from mouse stomach tissues. TrueScriptII Reverse Transcriptase kit (Sawady Technology Co., Ltd.) and oligo dT primer (dT) 30 were used for synthesizing cDNA. PCR was performed with 10 ng cDNA derived from RNA as a template, 10 pmol of primer, 0.5 U of EX-Taq polymerase (Takara Shuzo) and reaction buffers attached to EX-Taq polymerase, in a total volume of 20 μ l. The PCR was conducted under the following conditions: one cycle at 94.degree. C. for 5 minutes, 35 cycles at 95.degree. C. for 30 seconds, 62.degree. C. for 3 seconds, 72.degree. C. for 2 minutes, and finally one cycle at 72.degree. C. for 5 minutes. In addition, the validity of the sequences of PCR amplification products were confirmed by examining DNA sequences. PCR domains corresponding to each isoform of PTP ζ are PTP ζ -A type (nucleotideNo. 4861-5499), PTP ζ -B type (2321-2875), and PTP ζ -S type (4913-5704).

[0052] Western blotting for detecting PTP ζ in mouse stomachs was conducted as follows. With Tris buffer containing 1% NP-40 and protease inhibitors, gastric tissues were homogenized, and after the supernatant was obtained by centrifugation. Chondroitinase ABC treatment of the supernatant was carried out at 37.degree. C. for 1 hour. Further, proteins were separated by SDS-PAGE (6% acrylamide gel) and transferred to PVDF membrane by semi-dry transfer. In addition, rabbit anti-6B4 polyclonal antibody (1 μ g/ml) and peroxidase-labeled anti-rabbit antibody were used as secondary antibodies for immunostaining to detect PTP ζ . As a reaction substrate, ECL Plus reagent (Amersham Pharmacia) was used.

(B-3 Formation of gastric ulcer caused by VacA, a toxin of *Helicobacter pylori*)

[0053] After a mouse (female) of 4 weeks old was starved for 24 hours (fed water ad libitum), VacA, a toxin of *Helicobacter pylori* or saline was orally administered to the mouse with a sound. 48 hours later, gastric specimens were prepared and the surface of inside wall of stomach was observed by a stereomicroscope, and then pathologic speci-

mens were prepared by hematoxylen eosin staining and analyzed.

(B-4 Pathological evaluation of gastric ulcer formation caused by VacA)

5 **[0054]** Pathological evaluation of gastric ulcer formation caused by VacA was conducted in a following way. Epithelial damage score (EDS) was estimated according to the following scale: 1 = no lesion; 2 = disarray of columnar cells; 3 = diffuse microerosions and epithelium disaggregation; 4 = erosive lesion, denudation of basal membrane, and ulceration. At EDS level 4, gastric mucosal ulceration was considered to have occurred. For the inflammation score (IS), the amounts of inflammatory cells were graded as follows: 1 = none; 2 = scattered mononuclear and polymorphonuclear cells in the lamina propria and submucosa; 3 = definite increase in subepithelial areas of lamina propria; 4 = marked infiltration of lamina propria.

(B-5 Analyses of phosphorylation level of GIT1, a substrate molecule of PTP ζ and cell vacuolation in AZ-521 cells)

15 **[0055]** In order to analyze phosphorylation level of GIT1 (G protein-coupled receptor kinase-interactor 1) (PNAS, 98, 6593-6589, 2001), a substrate molecule of PTP ζ , and cell vacuolation in AZ-521 cells, uptake of neutral red and immunoprecipitation were conducted as follows. First, AZ-521 (Health Science Research Resources Bank), a cancerous cell strain derived from human stomach hypersensitive to VacA, was inoculated on a plate such that cell density was adjusted to be 350,000/cm² and cultured for 24 hours, and then added with acid-activated VacA and 100 ng/ml EGF. 30 minutes after the stimulation, cell vacuolation (uptake of neutral red) and tyrosine phosphorylation level of GIT1 (immunoprecipitation) were analyzed.

20 **[0056]** An uptake test of the pigment, neutral red, was conducted as follows. After components of the medium were removed, phosphate buffer containing 0.05% neutral red and 0.5% BSA was added and made the cells uptake it for 5 minutes. The cells were washed three times with phosphate buffer containing 0.5% BSA and then the intracellularly uptaken pigment was extracted with 70% ethanol containing 0.05% hydrochloric acid, and absorbance (540 nM) was measured.

25 **[0057]** Next, a tyrosine phosphorylation test of GIT1 was conducted as follows. 300 μ l of lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, 10 μ g/ml pepstatin A, 1 mM sodium orthovanadate, and 1 mM NaF) was added to 1.9×10^6 cells and the resulting mixture was left on ice for 30 minutes. After centrifuging the extract of the mixture, protein concentration of the supernatant was adjusted to be 100 μ g/ml. 25 μ g sample and 25 μ l Protein G Sepharose 4FF (Amersham Pharmacia) were mixed for 2 hours, and the supernatant wherein components nonspecifically adsorbing to gel was excluded was prepared. 2 μ l of rabbit anti-GIT1 antiserum was added to the supernatant, and 1 hour after the reaction, 25 μ l Protein G Sepharose 4FF was added and reaction was conducted for another 3 hours. After the reaction, beads were washed three times with lysis buffer, and then specifically adsorbing components were extracted with sample buffer of SDS-PAGE. This sample was separated by SDS-PAGE and subsequently transferred to PVDF membrane by semi-dry transfer, and used for immunoprecipitation. Anti-tyrosine phosphorylation mouse monoclonal antibody PY20 was used for the detection of tyrosine phosphorylation of GIT1 and goat anti-GIT1 antibody (Santa Cruz Biotechnology, Santa Cruz) was used for the detection of GIT1.

30 Example 2-2 [Results]

(Expression of PTP ζ in a mouse gastric epithelial cell layer)

35 **[0058]** Whether PTP ζ was expressed in the mouse gastric epithelial cell layer was examined by using rabbit polyclonal antibody which specifically recognizes the extracellular domain of PTP ζ , and the results are shown in Fig. 9 (see reference 3). As it can be seen from the wild-type mouse (Fig. 9A) and the PTP ζ -deficient mouse (Fig. 9B), it has been revealed for the first time that PTP ζ expresses in a gastric epithelial cell layer of a wild-type mouse. The reason why PTP ζ was originally thought to express only in central nervous system may be explained by the fact that the expression in stomach was too low in comparison to that in brain to be distinguished from the background. In the present invention, it was possible to set extremely hypersensitive immunostaining conditions as a result of the use of PTP ζ -deficient mice as negative controls.

(Expression of PTP ζ isoforms in a mouse gastric epithelial cell layer)

40 **[0059]** The result of RT-PCR confirmed the expression of all splice variants of PTP ζ -A type, PTP ζ -B type and PTP ζ -S type in gastric tissue (Fig. 10A). On the other hand, Western blot analysis indicated that receptor-type PTP ζ -B type was dominant as an actual protein component, and as electrophoretic mobility was not changed by chondroitinase ABC treatment, it was revealed that the PTP ζ -B type expressed in the stomach is not modified with chondroitin sulfate (Fig.

10B).

(Formation of ulcer in mouse gastric epidermis)

5 **[0060]** Formation of ulcer in mouse gastric epidermis at 48 hours after the administration of VacA was examined with a stereomicroscope. The results are shown in Fig. 11. As to wild-type mice that were administered with VacA, 3 out of 7 mice developed apparent ulcers in their stomachs (arrows, Fig. 11A), and in the stomachs of the other 4 mice, though it was not severe, formation of erosion or apparent disquamation of gastric epidermal layer was observed. On the contrary, in the stomachs of PTP ζ -deficient mice (n=7) that were orally administered with VacA, no significant damage was observed (Fig. 11B). Hematoxyline eosine staining of specimens of wild-type mice administered with VacA showed representative ulcers (arrows in Fig. 11C with low magnification and in Fig. 11D with high magnification), and the appearance of the ulcer was found to closely resemble that of human lesions caused by an infection with *Helicobacter pylori*. By contrast, as the PTP ζ -deficient mice were totally normal (Fig. 11E), it has been revealed for the first time at individual level that PTP ζ plays an indispensable role in the formation of ulcer caused by VacA. As to groups to which saline was administered, both wild-type (Fig. 11F) and PTP ζ -deficient mice were normal.

(Pathological evaluation of ulcer formation on mouse gastric epidermis)

20 **[0061]** The levels of epithelial damage score (EDS) and inflammation score (IS) were evaluated according to the method described in (B-4). Mean \pm SEM of EDS and IS are shown in Table 2. Epithelial damage and inflammation dependent to the dose of VacA were observed in wild-type mice (PTP ζ ^{+/+}), but no abnormality was observed in PTP ζ -deficient mice (PTP ζ ^{-/-}). In the Table, the results of Mann-Whitney's U test comparing to wild-type mice administered with saline are shown as #, P<0.05; ##, P<0.001, and to wild-type mice administered with VacA (0.5 mg/kg) as **, P<0.001. Further, "nd" means that analysis was not conducted.

Table 2

VacA (mg/kg)	n		EDS		IS	
	PTP ζ ^{+/+}	PTP ζ ^{-/-}	PTP ζ ^{+/+}	PTP ζ ^{-/-}	PTP ζ ^{+/+}	PTP ζ ^{-/-}
0	7	7	1.1 \pm 0.4	1.3 \pm 0.5	1.3 \pm 0.5	1.1 \pm 0.4
0.125	5	nd	1.8 \pm 0.8	nd	1.4 \pm 0.9	nd
0.250	5	nd	2.2 \pm 0.4#	nd	1.4 \pm 0.6	nd
0.500	10	10	3.8 \pm 0.4##	1.3 \pm 0.5**	3.6 \pm 0.5##	1.3 \pm 0.5**

(Analyses of phosphorylation level of GIT1, a substrate molecule of PTP ζ and cell vacuolation in AZ-521 cells)

40 **[0062]** Tyrosine phosphorylation of GIT1, a substrate molecule of PTP ζ , caused by stimuli with VacA was examined and the results are shown in Fig. 12. In VacA-hypersensitive strain AZ-521 cells, it was shown that tyrosine phosphorylation level of GIT1, a substrate molecule of PTP ζ decreased dose-dependently by stimulation with VacA (Fig. 12A), and that decrease in phosphorylation of GIT1 was correlated with uptake of neutral red by cells (index of vacuole formation) (Fig. 12B). Cytotoxicity of VacA was evaluated as cell vacuolation ability in vitro, and a possibility that VacA is involved in vacuole formation by increasing the activity of PTP ζ and by decreasing tyrosine phosphorylation of GIT1 has been shown.

Example 3 (Role of PTP ζ in gastric ulcer formation caused by pleiotrophin)

50 **[0063]** Pleiotrophin is a heparin-binding secretory protein of 18 kD, and known to be involved in neurotrophic factor-like activity, adhesion of neurons, elongation of process and angiogenesis (BioScience Jargon Library: "Cytokine, Growth Factor", Yodosha, p126-127, 1998). Further, pleiotrophin is known to bind to extracellular regions of PTP ζ (J. Biol. Chem. 271, 21446-21452, 1996) and alter the activity of PTP ζ (PNAS, 97, 2603-2608, 2000). Therefore, whether pleiotrophin is involved in the formation of gastric ulcer was examined.

55 Example 3-1 [Method]

[0064] The role of PTP ζ in gastric ulcer formation caused by pleiotrophin was examined as follows. After a mouse (female) of 4 weeks old was starved for 24 hours (fed water ad libitum), pleiotrophin (Peptide Institute, Inc.) was orally

administered to the mouse with a sound. 48 hours later, gastric specimens were prepared and the surface of inside wall of stomach was observed by a stereomicroscope, and then pathologic specimens were prepared by hematoxylen eosin staining and analyzed.

5 Example 3-2 [Results]

10 **[0065]** The level of epithelial damage on mouse gastric epithelium at 48 hours after the administration of pleiotrophin was examined under a stereomicroscope. The results are shown in Fig. 13. Profuse bleeding was observed in stomachs of wild-type mice administered with pleiotrophin (Fig. 13A). By contrast, no damage was observed in stomachs of PTP ζ ^{-/-} mice administered with pleiotrophin (Fig. 13B). As a result of hematoxylen eosin staining of specimens of wild-type mice administered with pleiotrophin, obvious damage was observed on gastric epithelium (Fig. 13C). On the other hand, PTP ζ -deficient mice were totally normal (Fig. 13D). These results revealed for the first time that ulcer was initiated by binding of PTP ζ and a ligand molecule. The involvement of pleiotrophin in gastric ulcer formation is a finding that has never been known, and it was revealed that PTP ζ -deficient mice were extremely useful as negative controls for the formation of gastric ulcer caused by pleiotrophin.

Industrial Applicability

20 **[0066]** The present invention has shown for the first time that PTP ζ is physiologically important in central monoamine pathway, particularly, in dopamine pathway. As pharmaceuticals that specifically adjust tyrosine phosphatase activity of PTP ζ have not been developed yet, there is a good chance that screenings of specific pharmaceuticals with the use of PTP ζ -deficient mice will lead to the development of a novel remedy for nervous affection.

25 **[0067]** Further, the present invention has histologically shown for the first time that PTP ζ is actually expressed in stomach, and has demonstrated at the level of individual mouse that PTP ζ is involved in the formation of gastric ulcer as a host receptor of VacA, a toxin secreted by *Helicobacter pylori*.

[0068] It is highly possible that these results will lead to the elucidation of onset mechanism of gastric ulcer caused by an infection with *Helicobacter pylori*, and the development of novel remedies for gastric ulcer and gastritis.

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SEQUENCE LISTING

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<110> JAPAN SCIENCE AND TECHNOLOGY CORPORATION

<120> Processes for screening PTP-zeta inhibitor or activator

<130> A011-12PCT

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Claims

- 55
1. A method for screening a PTP ζ inhibitor or activator wherein a subject material is administered to a non-human animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and a wild-type non-human animal, and PTP ζ activities in these non-human

animals are compared and evaluated.

2. The method for screening a PTP ζ inhibitor or activator according to claim 1, wherein the comparison and the evaluation of PTP ζ activity is the comparison and the evaluation of the function of central monoamine pathway.
3. The method for screening a PTP ζ inhibitor or activator according to claim 2, wherein the comparison and the evaluation of the function of central monoamine pathway is the comparison and the evaluation of changes in the level of central monoamine metabolism, sensitivity to a stimulant drug, the presence of dysfunction of mesolimbic dopamine pathway, level of acclimation to new circumstances, or stress-responsiveness.
4. The method for screening a PTP ζ inhibitor or activator according to claim 1, wherein the comparison and the evaluation of PTP ζ activity is the comparison and the evaluation of the level of binding to VacA, a toxin of *Helicobacter pylori*.
5. The method for screening a PTP ζ inhibitor or activator according to claim 1, wherein the comparison and the evaluation of PTP ζ activity is the comparison and the evaluation of the level of binding to pleiotrophin, a heparin-binding secretory protein.
6. The method for screening a PTP ζ inhibitor or activator according to any one of claims 1 to 5, wherein the non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome is purified by being backcrossed for 4 or more generations.
7. The method for screening a PTP ζ inhibitor or activator according to any one of claims 1 to 6, wherein the non-human animal is a mouse.
8. A PTP ζ inhibitor or activator obtained by the method for screening a proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor or activator according to any one of claims 1 to 7.
9. The PTP ζ inhibitor or activator according to claim 8, wherein the PTP ζ inhibitor or activator is a binding inhibitor between PTP ζ and VacA, a toxin of *Helicobacter pylori*.
10. The PTP ζ inhibitor or activator according to claim 8, wherein the PTP ζ inhibitor or activator is a binding inhibitor between PTP ζ and pleiotrophin, a heparin-binding secretory protein.
11. A remedy for dysfunction of central monoamine pathway containing the proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor or activator according to claim 8 as an active component.
12. A remedy for gastric ulcer or gastritis caused by *Helicobacter pylori* containing the proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor according to claim 8 or 9 as an active component.
13. A remedy for gastric ulcer or gastritis caused by pleiotrophin containing the proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) inhibitor according to claim 8 or 10 as an active component.
14. A non-human model animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and which is hyposensitive to a central stimulant drug (an addictive drug).
15. The non-human model animal according to claim 14, wherein the non-human animal is hyposensitive to a stimulant drug.
16. The non-human model animal according to claim 14 or 15, wherein the non-human animal whose function of gene DNA that encodes PTP α is deficient on its chromosome is purified by being backcrossed for 4 or more generations.
17. The non-human model animal according to any one of claims 14 to 16, wherein the non-human animal is a mouse.
18. A non-human model animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and which is hyposensitive to VacA, a toxin of *Helicobacter pylori*.

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19. A non-human model animal whose function of gene DNA that encodes proteoglycan-type receptor-type protein tyrosine phosphatase (PTP ζ) is deficient on its chromosome and which is hyposensitive to pleiotrophin, a heparin-binding secretory protein.

5 **20.** The non-human model animal according to claim 18 or 19, wherein the non-human animal whose function of gene DNA that encodes PTP ζ is deficient on its chromosome is purified by being backcrossed for 4 or more generations.

21. The non-human model animal according to any one of claims 18 to 20, wherein the non-human animal is a mouse.

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FIG. 1

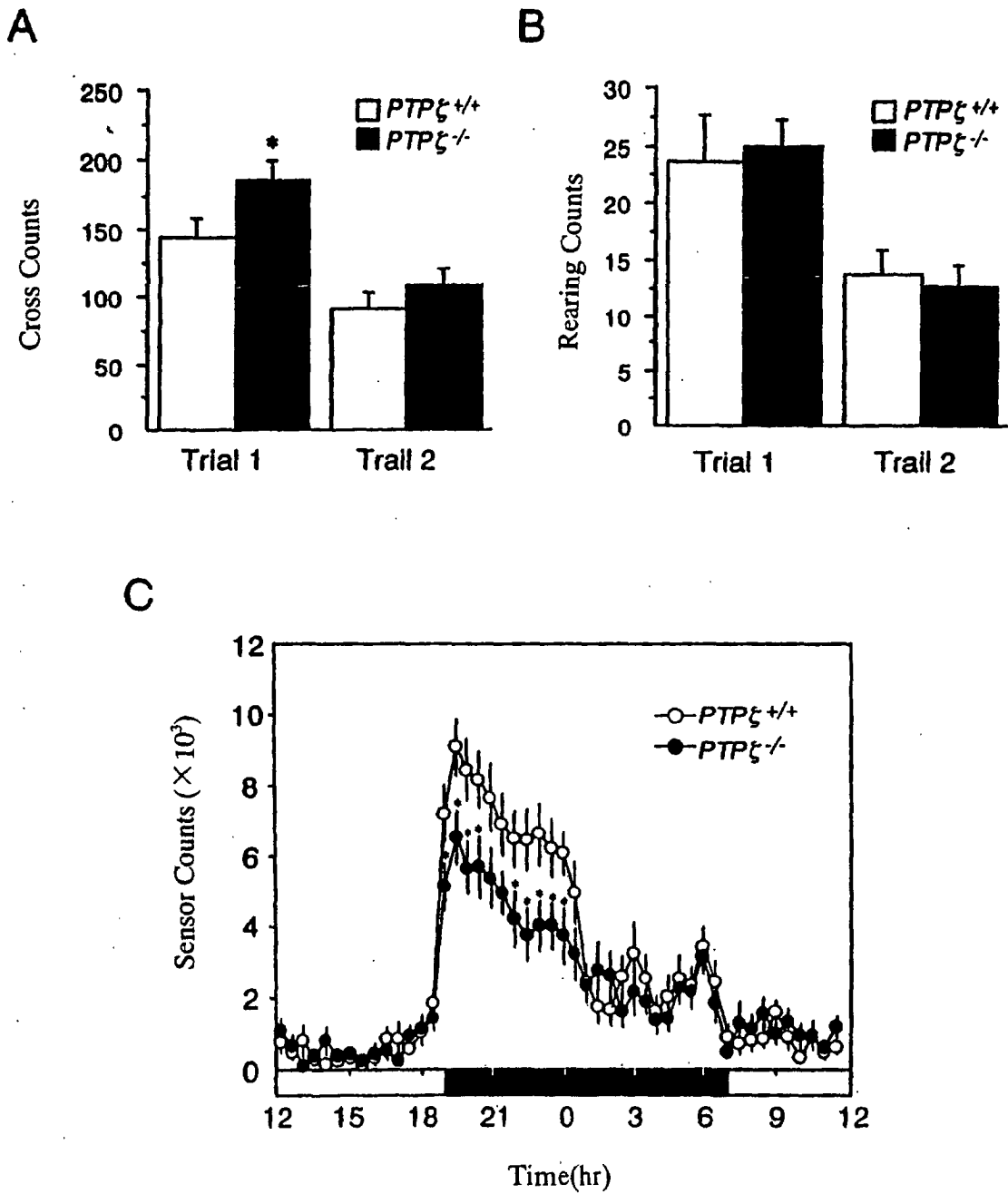


FIG. 2

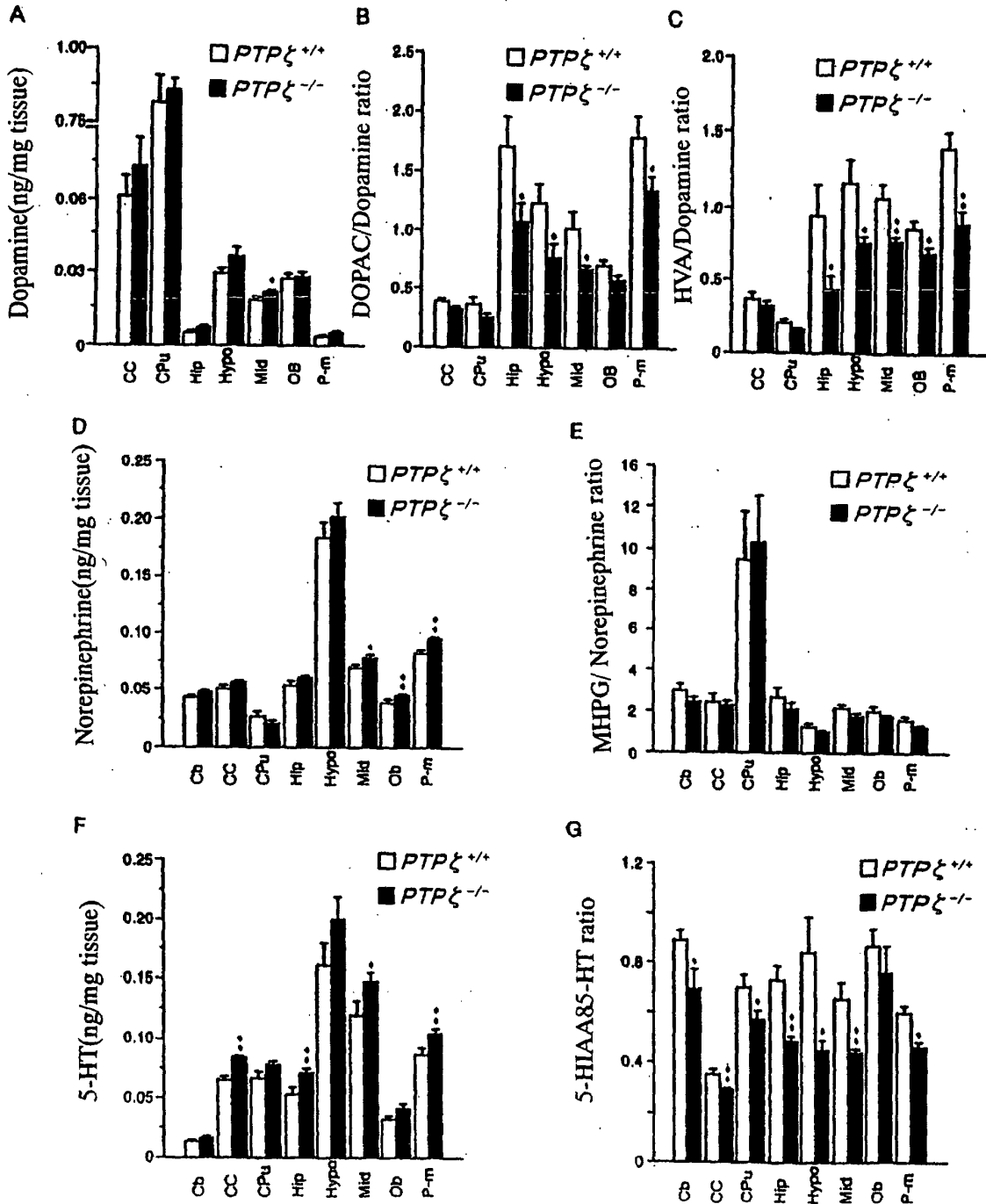


FIG. 3

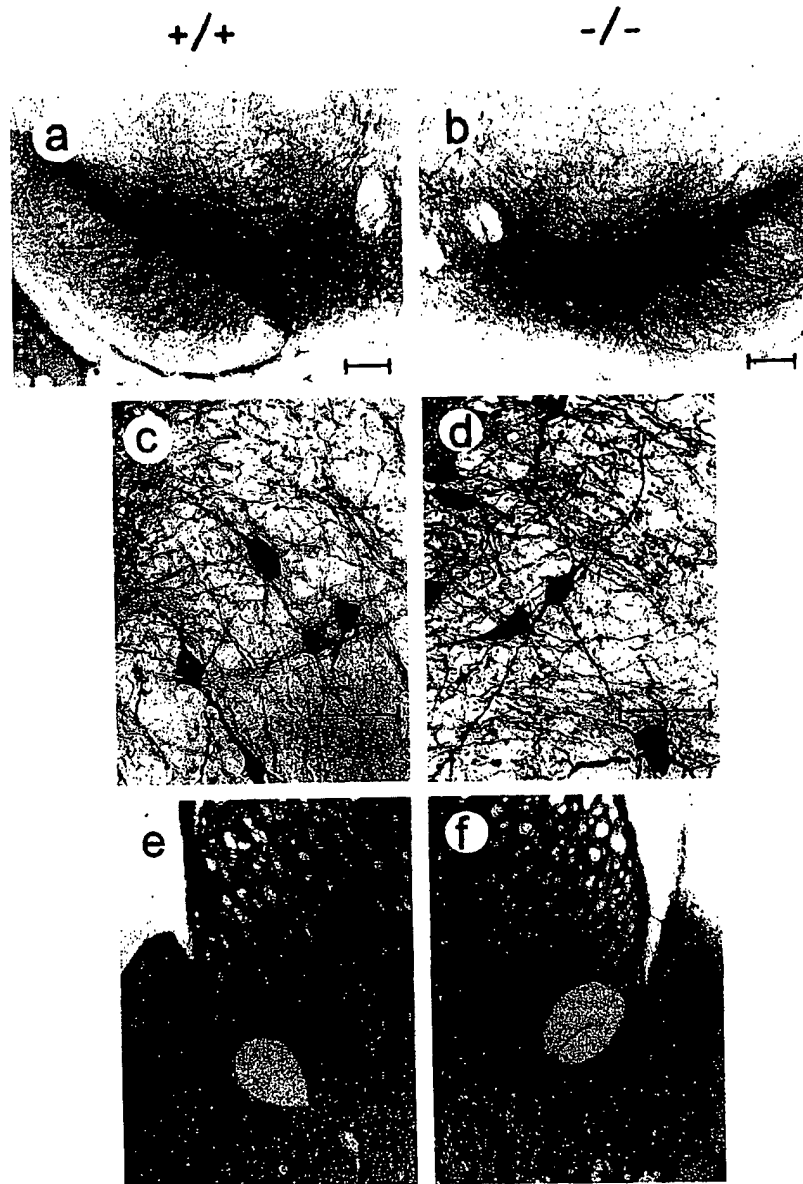
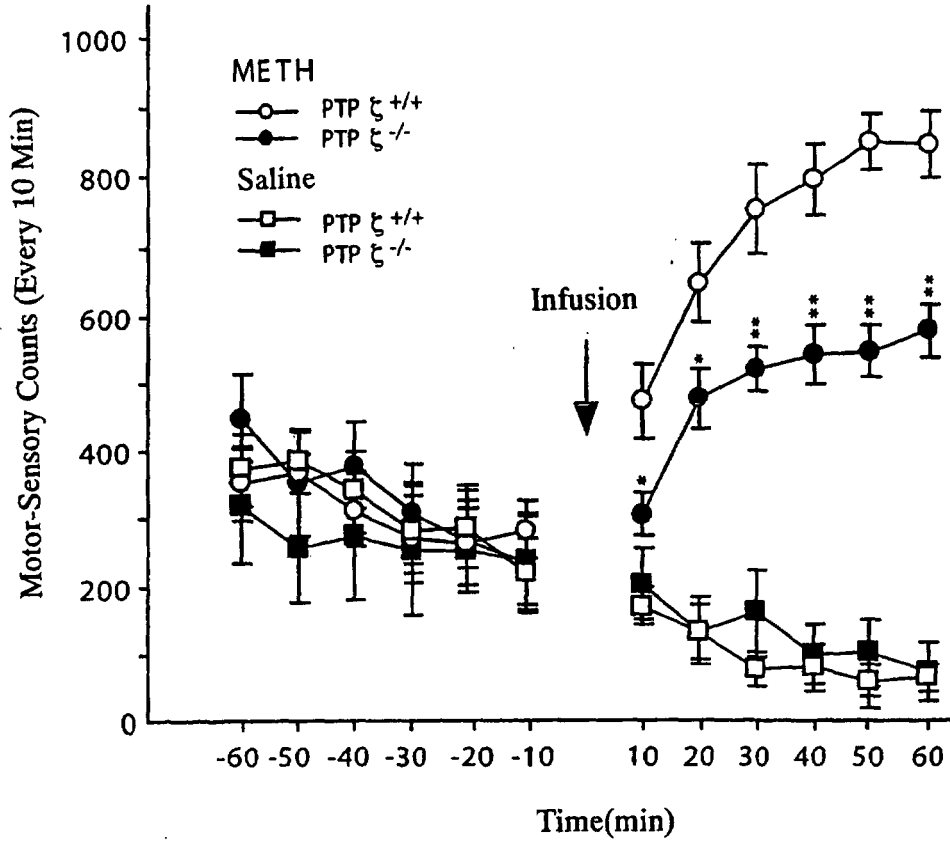


FIG. 4

A



B

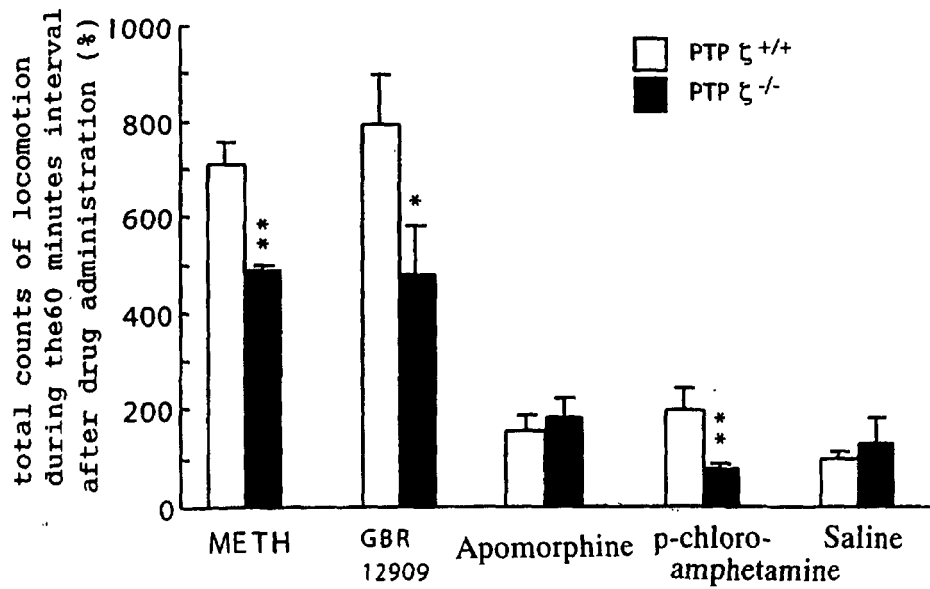


FIG. 5

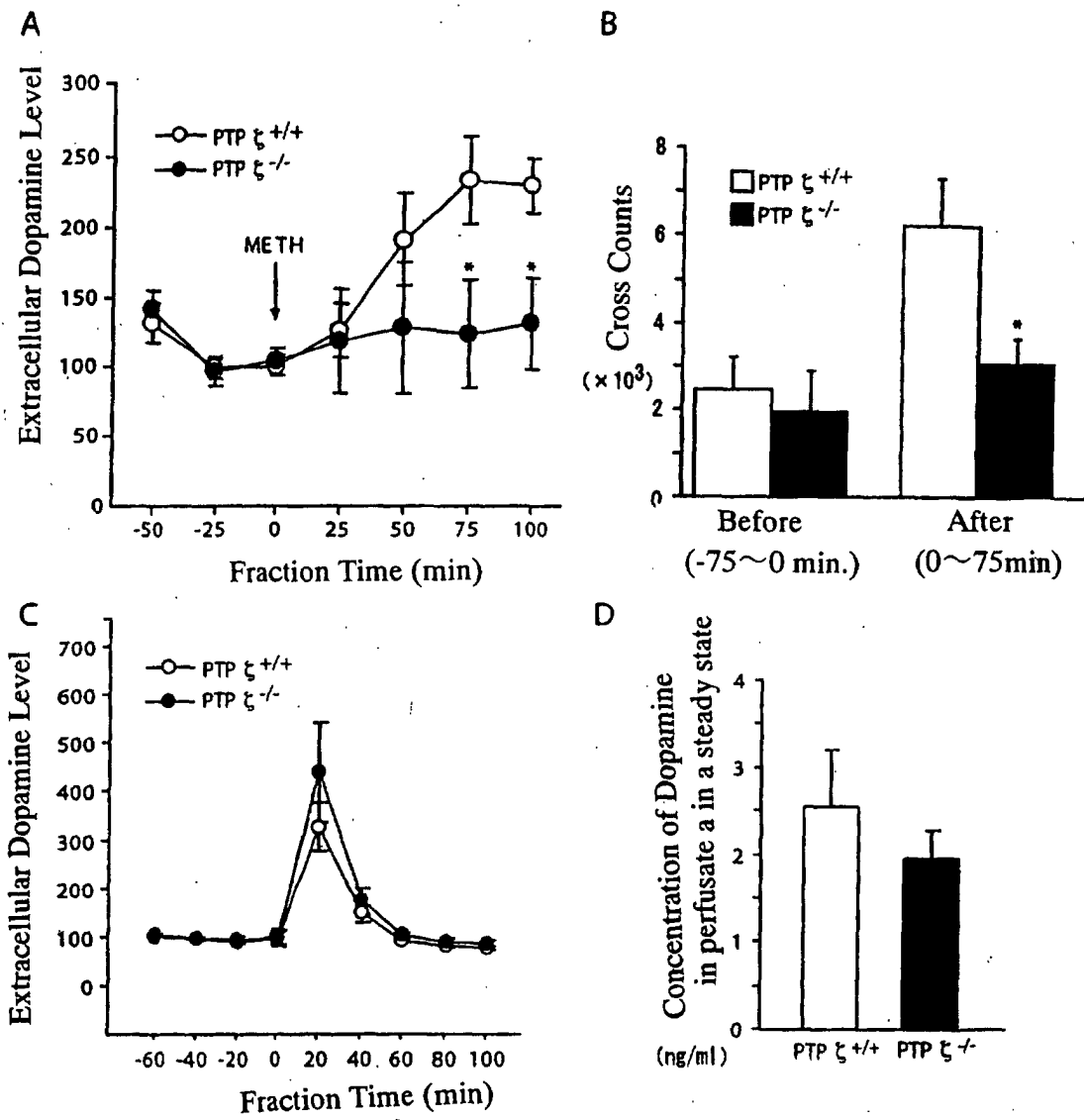
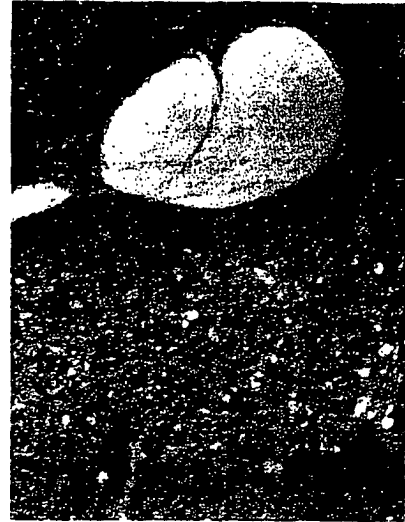


FIG. 6

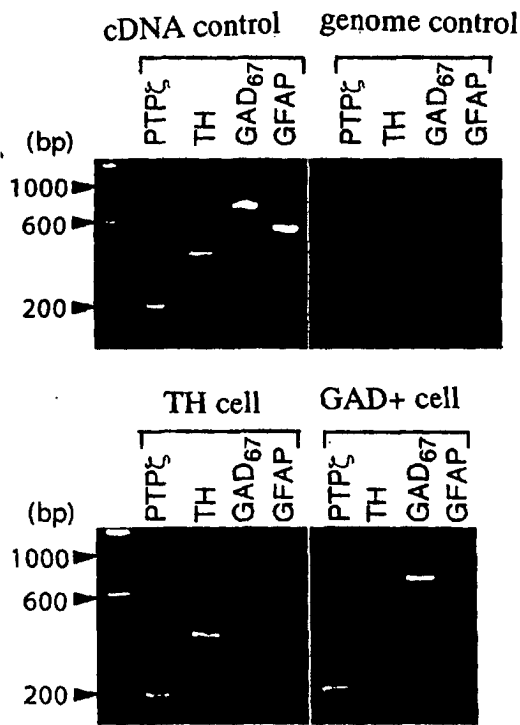
A



B



C



D

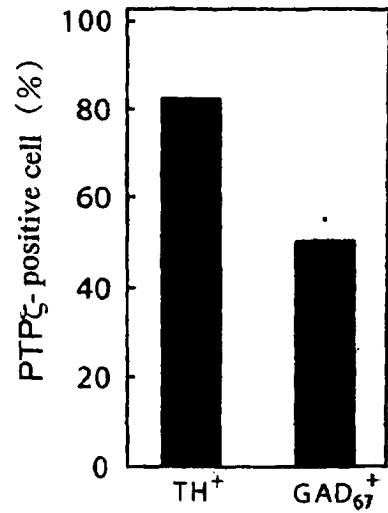


FIG. 7

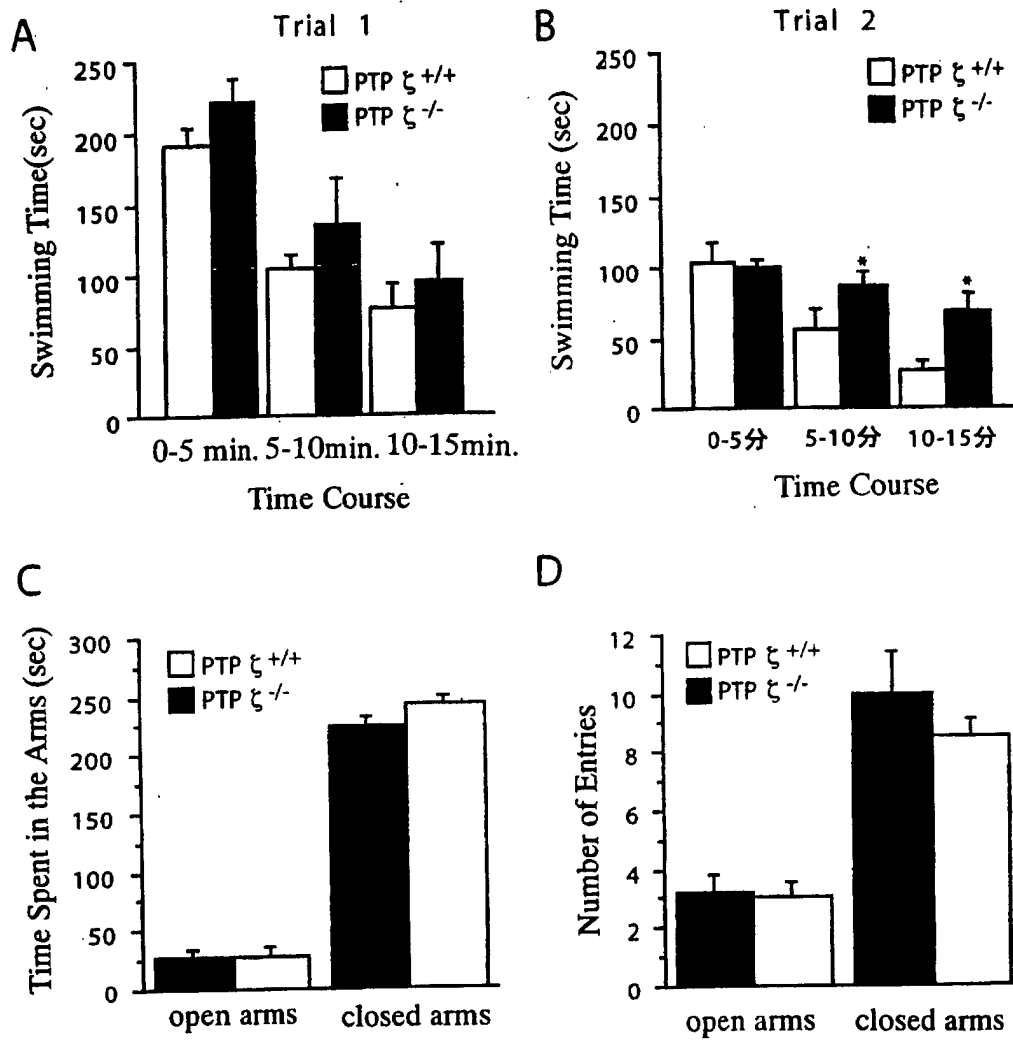


FIG. 8

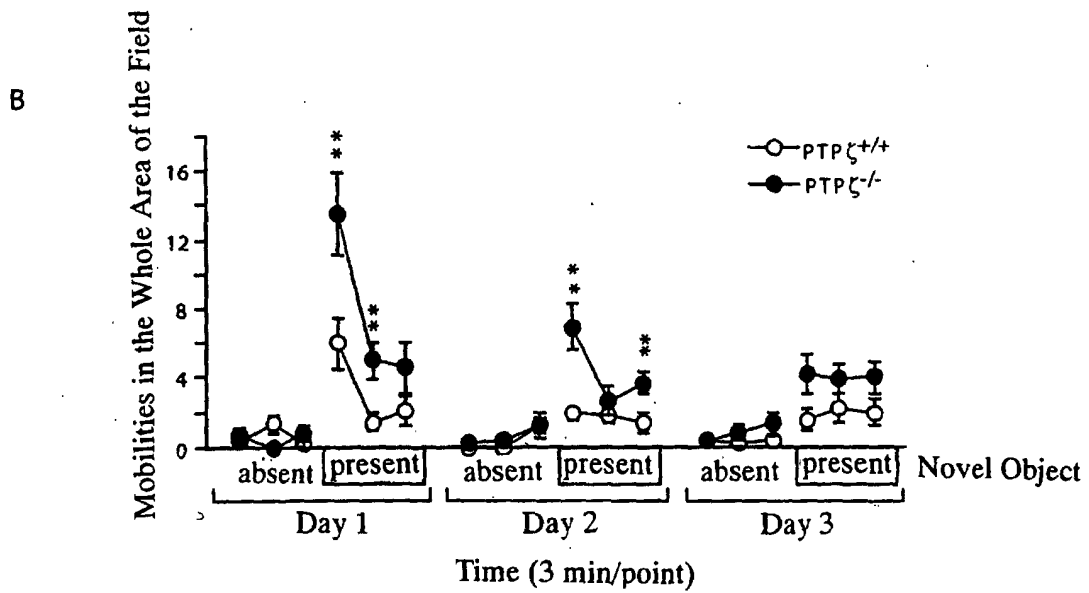
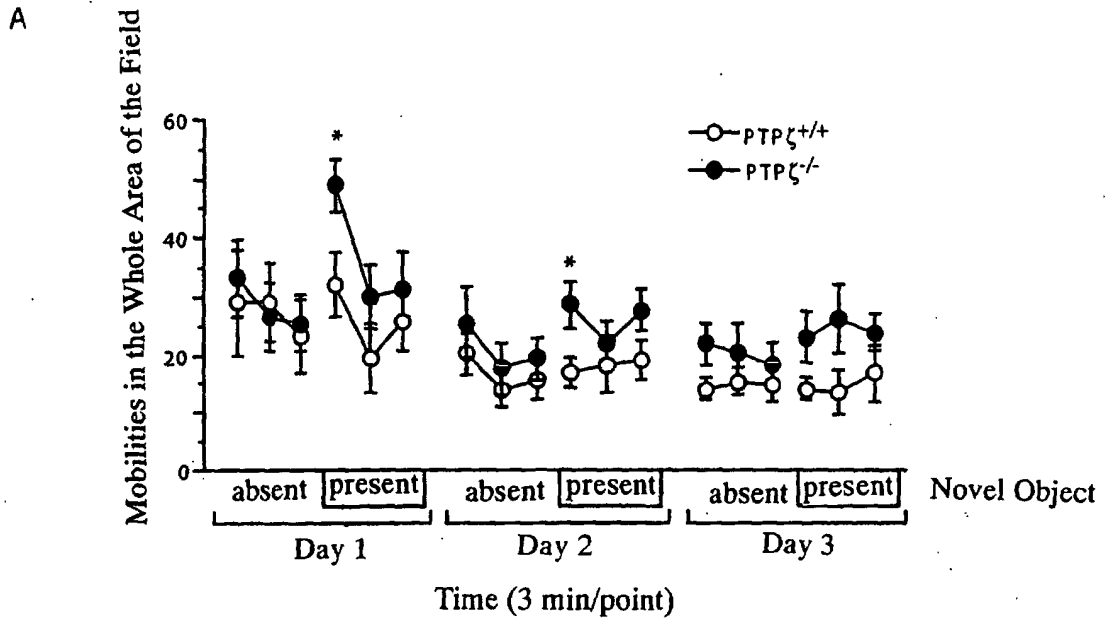
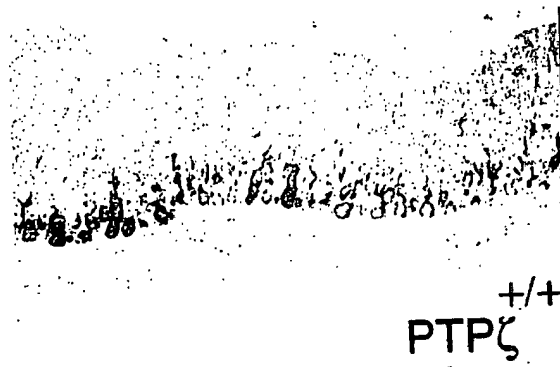


FIG. 9

A

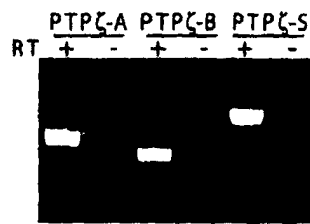


B



FIG. 10

A



B

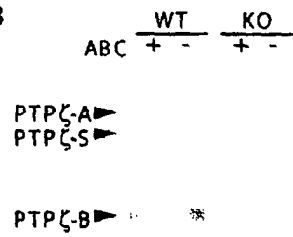


FIG. 11

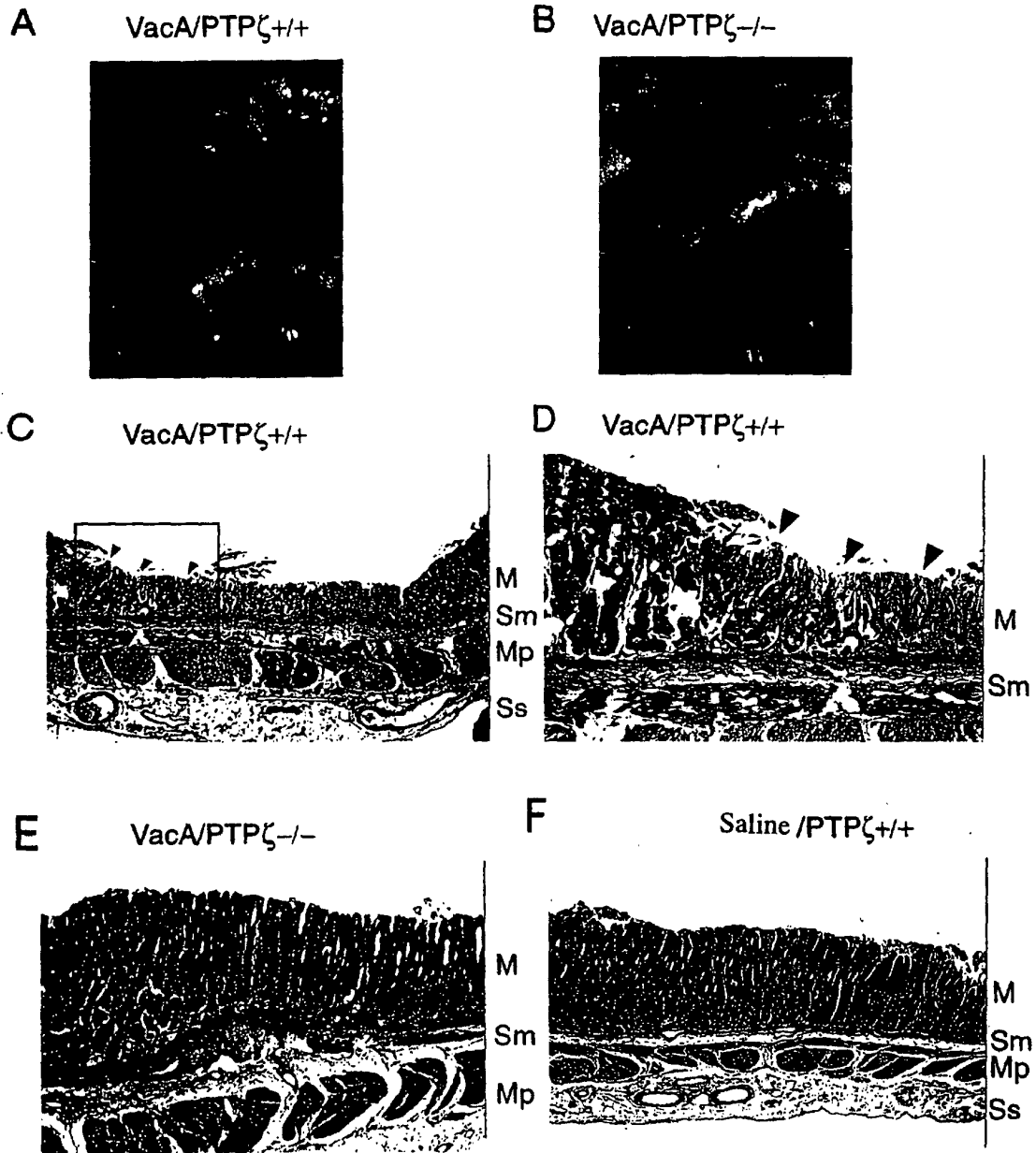
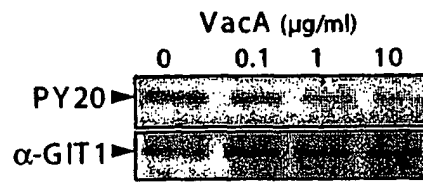


FIG. 12

A



B

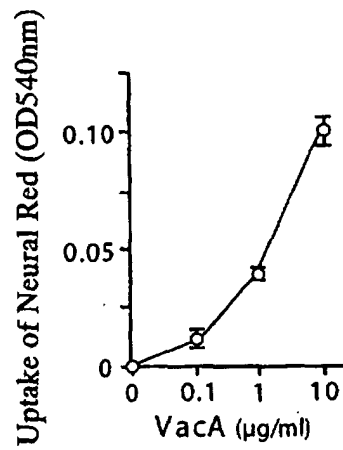
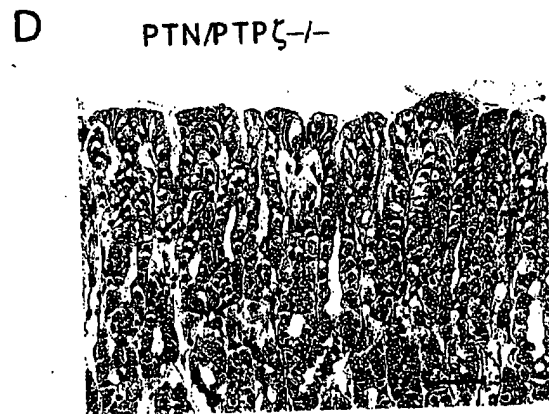
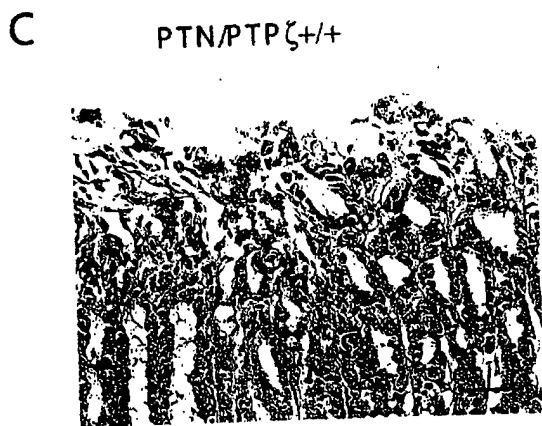
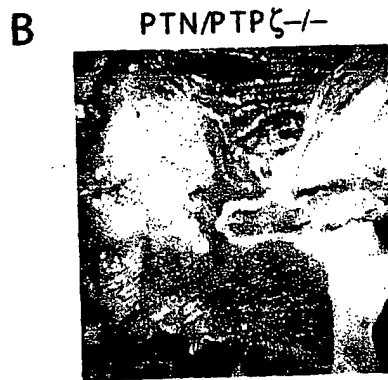
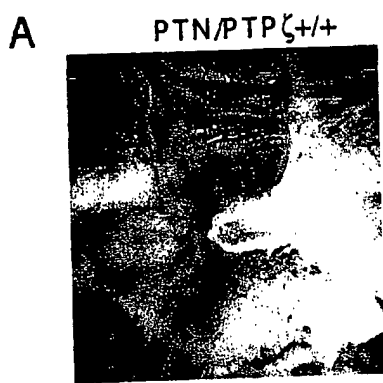


FIG. 13



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP01/06343

A. CLASSIFICATION OF SUBJECT MATTER Int.Cl. ⁷ C12N15/09, G01N33/50, 33/15, A01K67/027, A61K45/00, A61P25/00, 1/04		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) Int.Cl. ⁷ C12N15/09, G01N33/50, 33/15, A01K67/027		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) BIOSIS (DIALOG), WPI (DIALOG), JICST FILE (JOIS)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Takafumi SHINTANI et al., "Neurons as well as astrocytes express proteoglycan-type protein tyrosine phosphatase zeta/RPTPbeta: analysis of mice in which the PTPzeta/RPTPbeta gene was replaced with the LacZ gene", Neuroscience Letters, (1998), Vol.247, pages 135 to 138	1, 6-7, 14-21
Y		4-5
Y	Kinnosuke YASHIRO et al., "Activation of Helicobacter pylori VacA toxin by alkaline or acid conditions increases its binding to a 250-kDa receptor protein-tyrosine phosphatase beta", J. Biol. Chem., (1999), Vol.274, No.51, pages 36693 to 36699	4
Y	Nobuaki MAEDA et al., "6B4 proteoglycan/phosphacan, an extracellular variant of receptor-like protein-tyrosine phosphatase zeta/RPTPbeta, binds pleiotrophin/heparin-binding growth-associated molecule (HB-GAM)", J. Biol. Chem., (1996), Vol.271, No.35, pages 21446 to 21452	5
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family	
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 06 August, 2001 (06.08.01)	Date of mailing of the international search report 21 August, 2001 (21.08.01)	
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer	
Facsimile No.	Telephone No.	

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP01/06343

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Nobuaki MAEDA et al., "A receptor-like protein-tyrosine phosphatase PTPzeta/RPTPbeta binds a heparin-binding growth factor midkine. Involvement of arginine 78 of midkine in the high affinity binding to PTPzeta", J. Biol. Chem., (1999), Vol.274, No.18, pages 12474 to 12479	1-7, 14-21

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP01/06343

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. Claims Nos.: 8-13
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
- Concerning the "PTP ζ activity promoter or inhibitor" as set forth in the above claim, the description merely discloses a screening method but no substance obtained by the screening method. Even though the common technical knowledge at the point of the application is taken into consideration, it is completely unknown what particular compounds are involved in the scope thereof. Thus, the above claim is described in an extremely unclear manner. Therefore, no meaningful international search can be practiced on this claim.
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

- Remark on Protest** The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.