

(PROPOSED AMENDMENTS)

peptide fragment or derivative thereof
under conditions which will allow binding
to occur; and

5 (b) detecting any binding which has
occurred.

~~93. The method according to claim 92 which is
performed in vivo.~~

10 94. The method according to claim 92 performed in
vitro.

15 95. The method according to claim 92 in which the
disease or disorder of the nervous system is selected from
the group consisting of a tumor, a degenerative disease, a
retinal disease, and a sensory neuron disease.

20 96. The method according to claim 95 in which the
tumor is a neuroblastoma.

25 97. A method of treating a disease or disorder of
the nervous system comprising administering an effective
amount of BDNF protein, or a functionally active peptide
fragment or derivative thereof, to a patient.

30 98. The method according to claim 97 in which the
disease or disorder of the nervous system is a degenerative
disease.

~~99. The method according to claim 98 in which the
degenerative disease is retinal disease.~~

100. The method according to claim 97 in which the disease or disorder of the nervous system comprises damage to the nervous system.

5

101. The method according to claim 100 in which damage is caused by events selected from the group consisting of trauma, surgery, infarction, infection, and malignancy.

10

102. The method according to claim 97 in which the disease or disorder is caused by exposure to a toxic agent.

103. The method according to claim 97 in which the disease or disorder is caused by nutritional deficiency.

15

104. The method according to claim 94 in which the disorder comprises a congenital disorder of the retina.

20

105. A method of treating a disease or disorder of the nervous system comprising administering an effective amount of a combination of BDNF protein, or a functionally active peptide fragment or derivative thereof, and a second agent.

25

106. The method according to claim 105 in which the combination which combination exhibits synergism in a functional activity of BDNF to a patient.

30

107. The method of claim 105 in which the second agent is nerve growth factor.

35

108. The method of claim 105 in which the second agent is another member of the BDNF/NGF family.

109. The method according to claim 107 in which the disease or disorder is a degenerative disease.

5 110. The method according to claim 109 in which the degenerative disease is selected from the group consisting of Alzheimer's disease, Huntington's chorea, retinal disease and Parkinson's disease.

10 111. The method according to claim 105 in which the disease or disorder comprises damage to the nervous system.

15 112. The method according to claim 105 in which the disorder is damage caused by an event selected from the group consisting of trauma, surgery, infarction, infection, malignancy, and toxic agents.

20 113. The method according to claim 105 in which the disease or disorder comprises a disease or disorder of sensory neurons.

25 114. The method according to claim 105 in which the disease or disorder comprises a congenital disorder of the retina.

30 115. A method for isolating a gene which is a member of the brain derived neurotrophic factor/nerve growth factor gene family, but which encodes neither nerve growth factor nor brain derived neurotrophic factor, comprising:
(a) selecting, from among a diversity of nucleic acid sequences, those sequences that are homologous to both BDNF and NGF;

- (b) identifying, from among the nucleic acid sequences selected in (a), those sequences which contain sequences of about at least 6 contiguous nucleotides that are not homologous to NGF and BDNF; and
- (c) isolating the sequences identified in (b).

116. The method according to claim 115 in which the method of step (a) comprises using the polymerase chain reaction technique, in which a pair of oligonucleotide primers are used which are capable of hybridizing to homologous regions of BDNF and NGF DNAs, such that the sense strand oligonucleotide primer is capable of hybridizing to a first homologous DNA sequence of BDNF and NGF, and the antisense strand oligonucleotide primer is capable of hybridizing to a second homologous DNA sequence of BDNF and NGF.

117. The method according to claim 116 in which the sense strand oligonucleotide primer comprises a nucleic acid sequence substantially as depicted in Figure 1, which is selected from the group consisting of from about residue 587 to residue 616, from about residue 713 to residue 739, from about residue 764 to residue 781, from about residue 863 to residue 898, and sequences thereof; and the antisense strand oligonucleotide primer comprises a nucleic acid sequence substantially as depicted in Figure 1, which is (a) selected from the group consisting of from about residue 587 to about residue 616, from about residue 713 to about 739, from about residue 764 to about residue 781, from about residue 863 to about residue 898, and (b) is different from the sense strand primer.

118. A method for isolating a gene which is a member of the brain derived neurotrophic factor/nerve growth factor gene family, but which encodes neither nerve growth factor nor brain derived neurotrophic factor, comprising:

5 (a) selecting, from among a diversity of nucleic acid sequences, those sequences that are homologous to either BDNF or NGF and a gene encoding the amino acid sequence substantially as depicted in Figure 14, denoted as M3/4.

10 (b) identifying, from among the nucleic acid sequences selected in (a), those sequences which contain sequences of about at least 6 contiguous nucleotides that are not homologous to NGF and BDNF; and

15 (c) isolating the sequences identified in (b).

119. The method according to claim 118 in which the method of step (a) comprises using the polymerase chain reaction technique, in which a pair of oligonucleotide primers are used which are capable of hybridizing to homologous regions of either BDNF or NGF and a gene encoding the amino acid sequence substantially as depicted in Figure 14, denoted as M3/4, such that the sense strand oligonucleotide primer is capable of hybridizing to a first homologous DNA sequence of BDNF or NGF or the gene encoding the amino acid sequence denoted as M3/4, or, alternatively, Neurotrophin-3, or NT-3, and the antisense strand oligonucleotide primer is capable of hybridizing to a second homologous DNA sequence of BDNF and NGF or the gene encoding M3/4.

120. A method for increasing the levels of BDNF expressed in nervous system cells comprising exposing the nervous system cells to kainic acid or a related compound.

121. A method for increasing the levels of BDNF expressed in nervous system cells comprising exposing the nervous system cells to a non-NMDA receptor agonist.

5

122. A method for increasing the levels of NGF expressed in nervous system cells comprising exposing the nervous system cells to kainic acid or a related compound.

10

123. A method for increasing the levels of NGF expressed in nervous system cells comprising exposing the nervous system cells to a non-NMDA receptor agonist.

15

124. A method of promoting the survival of dopaminergic neurons comprising exposing the neurons to effective levels of BDNF.

20

125. A method of promoting the survival of cholinergic neurons comprising exposing the neurons to effective levels of BDNF.

25

126. The method according to claim 125 in which the cholinergic neurons are basal forebrain cholinergic neurons.

30

127. The method according to claim 125 in which the cholinergic neurons are septal cholinergic neurons.

128. A method of suppressing the proliferation of astroglial cells comprising exposing the astroglial cells to an effective amount of BDNF.

35

129. A method of inhibiting the uptake of gamma aminobutyric acid in neurons comprising exposing the neurons to an effective amount of BDNF.

5

130. A method of upregulating the expression of NGF receptor on a cell surface comprising exposing the cell to an effective amount of BDNF.

10

131. A method of preparing active BDNF from a less active precursor molecule comprising exposing the precursor molecule to an effective amount of endoproteinase Arg-C.

15

132. The method according to claim 131 in which an effective amount of endoproteinase Arg-C is endoproteinase Arg-C at a concentration of 0.1 unit per microliter.

20

133. An active form of BDNF produced by the method of claim 131.

134. An active form of BDNF produced by the method of claim 132.

25

135. The method according to claim 98 in which the degenerative disease is Parkinson's disease.

30

136. The method according to claim 98 in which the degenerative disease is Alzheimer's disease.

137. The method according to claim 105 in which the disorder is damage caused by a toxic agent.

35

138. The method according to claim 98 in which the degenerative disease is a "Parkinson-Plus" syndrome.

5 139. The method according to claim 138 in which the "Parkinson-Plus" syndrome is selected from the group consisting of Progressive Spranuclear Palsey (Steele-Richardson-Olszewski Syndrome), Olivopontocerebellar Atrophy, Shy-Drager Syndrome (Multiple Systems Atrophy), and Guamanian Parkinsonism-Dementia complex.

10

140. The method according to claim 135 in which symptoms of Parkinson's disease are caused by a toxic agent.

15

141. The method according to claim 99 in which retinal disease is caused by damage to the optic nerve.

20

142. A method for increasing the levels of BDNF expressed in nervous system cells comprising exposing the nervous system cells to carbachol.

25

143. A method for increasing the levels of BDNF expressed in nervous system cells comprising exposing the nervous system cells to histamine.

30

144. A method for increasing the levels of BDNF expressed in nervous system cells comprising exposing the nervous system cells to bradykinin.

35

145. A method for increasing the levels of NGF expressed in nervous system cells comprising exposing the nervous system cells to carbachol.

36

BRAIN DERIVED NEUROTROPHIC FACTORAbstract

The present invention relates to nucleic acid sequences encoding brain derived neurotrophic factor (BDNF), as well as BDNF protein produced in quantity using these nucleic acid sequences, as well as fragments and derivatives thereof. In addition, the invention
5 relates to pharmacologic compositions and therapeutic uses of BDNF, having provided, for the first time, the means to generate sufficient quantities of substantially pure BDNF for clinical use. In a specific embodiment, BDNF may be used to promote the survival of substantia nigra dopaminergic neurons and basal
10 forebrain cholinergic neurons, thereby providing a method for treating, respectively, Parkinson's disease and Alzheimer's disease. The invention also relates to antibodies directed toward BDNF or fragments thereof, having provided a method for generating sufficient immunogen. Further, the invention provides
15 a method for identifying and isolating additional members of the BDNF/NGF gene family.

Figure 29