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(54) Title: METHOD OF TREATING COGNITIVE IMPAIRMENT AND COMPOUNDS FOR USE THEREIN

(57) Abstract: The present invention relates to the treatment, prevention or amelioration of a cognitive impairment in a subject comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist, preferably lamotrigine.
Title: Method of treating cognitive impairment and compounds for use therein

FIELD OF THE INVENTION

The present invention is in the field of medical treatment. In particular, the present invention is in the field of treating, preventing or ameliorating cognitive impairment in a subject, such as for example associated with the disorder neurofibromatosis type I. The present invention relates to a method of treating, preventing or ameliorating cognitive impairment in a subject. The invention further relates to HCN channel agonist for use in treating cognitive impairment in a subject.

BACKGROUND OF THE INVENTION

Neurofibromatosis type I (NF1) is a common autosomal dominant disorder, caused by heterozygous loss of function mutations within the NF1 gene. NF1 has an incidence of 1 in 3000, half of which are de novo cases. The disease is characterized by progressive neurocutaneous manifestations, including café au lait macules, axillary freckling, neurofibromas, and Lisch nodules. However, the most frequent complication of NF1 in children is cognitive impairments, which are characterized by a low-average IQ, and problems with visuospatial skills, memory, language, executive functioning, and attention. Because of these problems, up to 75% of children with NF1 have learning disabilities, and most children need additional support in the form of special education or remedial teaching. The cognitive phenotype is recapitulated in mice heterozygous for a null mutation of the Nf1 gene (Nf1+/-), as these mice show impairments in visual-spatial learning, working memory, attention, and motor performance (Shilyansky et al., 2010, Proc. Nat. Acad. Sci. 107, 13141–13146) (Costa et al., 2002, Nature 415, 526–530) (Silva et al., 2001, Clin Neurosci Res 1, 187–193) In addition these mice show impaired
hippocampal and cortical synaptic plasticity, which appears to be caused by ERK-mediated increase in synaptic GABA release from interneurons (Costa et al., 2002, supra; Shilyansky et al., 2010, Annu Rev Neurosci. 33, 221-43; Li et al., 2005, Curr Biol 15, 1961–1967; Cui et al., 2008, Cell 135, 549–560). The NF1 gene encodes neurofibromin, a large 2818 amino-acid protein containing a GTPase-activating protein (GAP) related domain, which serves to inactivate RAS. However, large regions of the NF1 coding sequence have been conserved across millions of years of evolution from Drosophila to man, suggesting significant functions outside the GAP related domain. In Drosophila, neurofibromin can function to modulate adenylate cyclase activity, and this function has been demonstrated in mammalian cell systems.

There are two major alternatively spliced exons of the NF1 gene in the adult brain: exon 9a, and 23a. Alternative isoform NF1-exon 23a (NF1 type II) is highly expressed in astrocytes. This isoform has decreased RAS-GAP activity, and mice lacking exon 23a (NF1<sup>23a-/-</sup> mice) show specific hippocampal learning impairments. Interestingly, expression of the alternative isoform NF1-exon 9a is exclusively restricted to neurons of the central nervous system. Exon 9a encodes only 10 amino acids, and is located in the amino terminal region of neurofibromin. NF1-exon 9a expression is enriched in the septum, striatum, cortex, and hippocampus beginning in the first week of postnatal life. However, the importance of this neuronal isoform is unclear, in particular since exon 9a resides well beyond the GAP related domain.

The present inventors produced a mouse model of NF1 which lacks a neuron specific isoform of NF1-9A. It was shown that mice lacking exon 9a have spatial learning and hippocampal plasticity deficits, which are caused by an increase in GABA-mediated inhibition. In addition, through a proteomics screen, the inventors showed that there is a down-regulation of HCN current in pyramidal cells and inhibitory interneurons which is associated with increased in intrinsic excitability of hippocampal neurons and GABAergic output on pyramidal neurons. The inventors subsequently showed that
applying HCN antagonist to wild-type mice mimics the NF1 phenotype, whereas applying an HCN agonist to the mutant NF1-9A mouse rescues both the electrophysiological phenotype as well as the learning deficits.

5 SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist.

In a preferred embodiment of a method of the invention as described above, said subject is suffering from a developmental intellectual disability, preferably impaired spatial learning and memory. Preferably the said developmental intellectual disability is due to aberrant inhibition of excitatory neurons in the brain, i.e., wherein the neurological or psychiatric impairment is the result of increased neuronal inhibition due to increased excitability of the interneurons.

These disorders include but or not limited to disorders that are the result of chromosome abnormalities or genetic syndromes, including, but not limited to Down syndrome and Neurofibromatosis type I (NF1), preferably NF1. In fact, any cognitive or psychiatric impairment that is the result from increased neuronal inhibition that can be ameliorated by stimulating HCN channel function, is included as an embodiment in aspects of this invention. The skilled person will understand when such a disorder is the result of increased inhibition, and whether the use of a HCN channel agonist will ameliorate or treat the condition as disclosed herein.

In yet another preferred embodiment of a method of the invention as described above, said cognitive impairment is characterized by learning disabilities, in particular patients are characterized by a low-average IQ, and
by having problems with visuospatial skills, memory, language, executive functioning, and attention.

In still another preferred embodiment of a method of the invention as described above, said subject is between the age of 1 and 18 years, preferably 2-12 years.

In yet another preferred embodiment of a method of the invention as described above, said HCN channel agonist is administered in a dosage of 0.1-100 mg/kg daily, preferably 5-15 mg/kg twice daily, preferably dosed between two-three hours before a cognitive training session.

In another preferred embodiment of a method of the invention said administration is done by a route selected from oral, sublingual, pulmonary, nasal, intramuscular, intradermal, rectal, subcutaneous, intraperitoneal and intravenous. Most preferably, the administration is done by the oral route.

In another preferred embodiment of a method of the invention, said HCN channel agonist is administered in a pharmaceutically acceptable carrier.

In yet another preferred embodiment of a method of the invention, said HCN channel agonist is selected from the group consisting of phenyltriazines, GABA analogs, (substituted) benzazepines, peptide neurotransmitters and (substituted) benzamides.

In yet another preferred embodiment of a method of the invention, said HCN channel agonist is lamotrigine, or an analogue, derivative and/or pharmaceutically acceptable salt thereof.

In still another preferred embodiment of a method of the invention, said lamotrigine analogue, derivative and/or pharmaceutically acceptable salt thereof up-regulates the HCN channel-mediated non-selective cationic current (Ih) in inhibitory interneurons of an Nf1<sup>+/−</sup> and/or an Nf1<sup>9a+/9a−</sup> mouse. Preferably, said inhibitory interneurons are parvalbumin immune-positive (PV<sup>+</sup>) hippocampal inhibitory neurons and/or somatostatin positive Oriens-lacunosum moleculare (O-LM) interneurons.
Method according to any one of the preceding claims, wherein said lamotrigine analogue, derivative and/or pharmaceutically acceptable salt thereof up-regulates the HCN channel-mediated non-selective cationic current (Ih) in of an NFI+/− and/or an NFI9a+/− mouse.

In another aspect, the present invention provides a HCN channel agonist, preferably selected from the group consisting of phenyltriazines, GABA analogs, (substituted) benzazepines, peptide neurotransmitters and (substituted) benzamides, more preferably selected from lamotrigine, or an analogue, derivative and/or pharmaceutically acceptable salt thereof, for use in treating cognitive impairment in a subject.

In a preferred embodiment of a HCN channel agonist for use according to the present invention as described above, said subject is suffering from a developmental intellectual disability, preferably impaired spatial learning and memory. Preferably the said developmental intellectual disability is due to aberrant inhibition of excitatory neurons in the brain, i.e., wherein the neurological or psychiatric impairment is the result of increased neuronal inhibition due to increased excitability of the interneurons.

These disorders include but or not limited to disorders that are the result of chromosome abnormalities or genetic syndromes, including, but not limited to Down syndrome and Neurofibromatosis type I (NF1), preferably NF1. In fact, any cognitive or psychiatric impairment that is the result from increased neuronal inhibition that can be ameliorated by stimulating HCN channel function is included as an embodiment in aspects of this invention.

In yet another preferred embodiment of a HCN channel agonist for use according to the present invention, said cognitive impairment is characterized by learning disabilities, in particular patients are characterized by a low-average IQ, and by having problems with visuospatial skills, memory, language, executive functioning, and attention.
In a preferred embodiment of a HCN channel agonist for use according to the present invention, said subject is a subject suffering from Neurofibromatosis type I (NF1).

In still another preferred embodiment of a HCN channel agonist for use according to the present invention, said subject is between the age of 1 and 18 years, preferably 2-12 years.

In another preferred embodiment of a HCN channel agonist for use according to the present invention, said HCN channel agonist is administered in a dosage of 0.1-100 mg/kg daily, preferably 5-15 mg/kg twice daily, preferably dosed between two-three hours before a cognitive training session.

In a preferred embodiment of a HCN channel agonist for use according to the present invention, said lamotrigine analogue, derivative and/or pharmaceutically acceptable salt thereof up-regulates the HCN channel-mediated non-selective cationic current (Iₙ) in an inhibitory neuron (interneuron) of an Nfi1+/− and/or an Nfi1⁹α/⁹α mouse. A particularly preferred embodiment is lamotrigine.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates co-immunoprecipitation of HCN1 with NF1 antibody in hippocampal lysates. The specificity of the HCN1 antibody was verified by taking a HCN-knock-out lysate as a control.

Figure 2 illustrates that Iₙ is down-regulated in PV expressing interneurons of Nfi1⁹α/⁹α mice. Representative voltage clamp recordings of Iₙ currents in a PV cell (A) and a pyramidal cell (E). Iₙ was evoked by 1-s hyperpolarizing voltage steps from holding potential of -50 to -120 mV in 10 mV increments and followed by a final step to -120 mV to obtain tail currents. ZD7288-sensitive currents obtained by subtracting currents before and after bath application of ZD7288 (30 µM). The current-voltage (I-V) relation of Iₙ obtained by plotting steady-state currents showed that a significantly smaller amplitude of Iₙ was activated in the PV cells from Nfi1⁹α/⁹α than those from
wild-type mice ($F_{1,16}=6.50, P<0.05$). Voltage dependence of $I_h$ activation was determined by plotting normalized tail current amplitude as a function of voltage and then fit with Boltzmann function. (C) The activation curve was shifted to hyperpolarized potentials in the PV cells from $NfI^{9a^*/9a^*}$ mice and the $V_{1/2}$ of these fits in PV cells from $NfI^{9a^*/9a^*}$ mice was $-88.4 \pm 1.9$ mV ($n=10$), compared to $-81.2 \pm 1.9$ mV ($n=7, P=0.012$) in control cells. The current-voltage relation (F) and the voltage dependence of $I_h$ activation measured from pyramidal neurons in $NfI^{9a^*/9a^*}$ was not different than from those obtained in wild-type. Typical responses of a PV cell (D, inset) and a pyramidal cell (H, inset) to a depolarizing current injection. (D) The mean number of action potentials generated in response to depolarizing current pulses in the PV cells from $NfI^{9a^*/9a^*}$ were significantly higher compared to wild-type controls ($F_{1,22}=12.32, P=0.04$). (H) There was no change in the average number of action potentials per depolarizing current pulse recorded in pyramidal cells between two groups.

Figure 3 illustrates that pharmacological block of HCN channels in wild-type mice mimics the enhanced inhibition as observed in $NfI^{9a^*/9a^*}$ mice. Representative traces of sIPSCs (top) and cumulative distributions of sIPSC inter-event intervals (bottom) recorded in wild-type (A) and $NfI^{9a^*/9a^*}$ neurons (B) before and 10 min after bath application of ZD7288 (30 µM). ZD7288 caused a significant shift towards shorter inter-event intervals in both groups (Kolmogorov-Smirnov test, $P<0.05$), indicative of an increase in sIPSCs frequency. (C) Group data showing an increase in mIPSC frequency in both groups (wt: $5.6 \pm 0.5$ Hz versus $7.5 \pm 0.7$ Hz, $n=9, p=0.001$ and in $NfI^{9a^*/9a^*}$ mice: $5.3 \pm 0.7$ Hz versus $6.8 \pm 0.8$ Hz, $n=12, p=0.001$ before and after ZD7288 respectively). (D) Application of ZD7288 had no effect on the amplitude of sIPSCs.

Figure 4 illustrates that up-regulates of $I_h$ by lamotrigine reduces the excitability of PV cells and rescues the LTP deficit in $NfI^{9a^*/9a^*}$ mice. (A) The current-voltage relation of $I_h$ in PV cells showing that the magnitude of $I_h$
was significantly increased by application of lamotrigine (50 μM) in Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice (F<sub>1,21</sub>=9.91, P<0.05). (B) The Ih activation curve was shifted to more depolarized potentials in PV cells from Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice in the presence of lamotrigine. V<sub>1/2</sub> for Ih in PV cells from Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice in the presence of lamotrigine was -80.9±2.0 mV (n=9) which was significantly different from that obtained in control condition in Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice (-88.4±1.9 mV, P=0.007) and reaches the wild-type level (dashed line). (C) The effect of increased Ih on firing rate as the percentage of control showed that increasing Ih by lamotrigine decreased firing rates over the entire range of current injections. The largest effect was found with the smallest current injections (52±8% in Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice and 61±7% in wild-type), indicating that Ih most strongly affects firing rates at membrane potentials that are relatively close to resting membrane potential. (D) LTP at Schaffer Collateral synapses was significantly reduced in vehicle-treated slices from Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice compared with that in wild-type mice (123.1±5.2, n=9 vs 143.9±5.9, n=6; P=0.007). (E) lamotrigine rescued the LTP deficit at CA3-CA1 synapses in Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> mice. In the presence of lamotrigine, LTP was 137.5 ± 4.4 (n=16) and 127.8±4.3 (n=10) in Nf1<sup>3a<sup>r<sup>+/3a<sup>r<sup>−</sup> and wild-type mice, respectively.

Figure 5 illustrates that Ih is also down-regulated in PV expressing interneurons of Nf1<sup>+/−</sup> mice. (A) Ih was evoked by 1-s hyperpolarizing voltage steps from holding potential of -50 to -120 mV in 10 mV increments, followed by a final step to -120 mV to obtain tail currents. The ZD7288-sensitive current was obtained by subtracting current before and after bath application of ZD7288. (B) Amplitude of Ih was significantly smaller in PV cells from Nf1<sup>+/−</sup> as compared to those obtained from wild-type mouse (F<sub>1,21</sub>=11.51, P<0.001). (C) Voltage dependence of Ih activation was shifted toward more hyperpolarized potentials in the PV cells from Nf1<sup>+/−</sup> mice. Based on sigmoidal fits to activation values, V<sub>1/2</sub> was significantly hyperpolarized in Nf1<sup>+/−</sup> mice (-89.7±1.7 mV, n=7) compared with wild-types (-84.7± 1.1 mV, n=7, P=0.009). Ih evoked in pyramidal cells using the same voltage-clamp protocol did not
show any difference in $I_h$ amplitude (E) and the voltage dependence of $I_h$ activation (F) between $Nf1^{+/−}$ and wild-types. (D) The mean number of action potentials generated in response to depolarizing current pulses in the PV cells from $Nf1^{+/−}$ were significantly higher compared to wild-type controls ($F_{1,20} = 10.13$, $P = 0.03$). (G) There was no change in the average number of action potentials per depolarizing current pulse recorded in pyramidal cells between two groups.

Figure 6 illustrates that administration of lamotrigine improves spatial learning of $Nf1^{3a^{+/−}3a^{+/−}}$ mice (A) and $Nf1^{+/−}$ mice (B) in the Morris watermaze without any significant effect on the performance of wild-type littermates. Learning is represented as time searched in the target quadrant in which the platform was previously located (black column) compared to the average time spent in other quadrants. (A) $Nf1^{3a^{+/−}3a^{+/−}}$ mice showed a significant genotype by drug treatment interaction (two-way ANOVA, $F_{1,40} = 5.949$, $P = 0.019$) for the time in the target quadrant. Post hoc analysis indicates that lamotrigine-treated $Nf1^{3a^{+/−}3a^{+/−}}$ mice (n=12) spent more time searching in the target quadrant than vehicle-treated $Nf1^{3a^{+/−}3a^{+/−}}$ mice (n=9). There was no significant difference between lamotrigine-treated (n=14) and vehicle-treated (n=12) wild-type mice ($P > 0.05$). (B) Vehicle-treated $Nf1^{+/−}$ mice also showed a subtle learning deficit which was reversed by lamotrigine. There was a significant genotype by drug treatment interaction (two-way ANOVA, $F_{1,40} = 5.188$, $P = 0.028$) for the time in the target quadrant. lamotrigine treatment significantly increased the time spent in the target quadrant in lamotrigine-treated $Nf1^{+/−}$ mice (n=10) as compared with vehicle-treated $Nf1^{+/−}$ mice (n=9). There was no significant difference between lamotrigine-treated (n=14) and vehicle-treated (n=12) wild-type $Nf1^{+/−}$ mice ($P > 0.05$). (C) Illustrates that accelerating rotarod coordination is significantly improved in lamotrigine treated $Nf1^{+/−}$ mice compared to vehicle-treated $Nf1^{+/−}$ mice. No effect of lamotrigine was observed in wild-type littermate control mice.
DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the terms "treating" and "treatment" refer to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a medical conditions or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of the disorder or impairment and related conditions and includes palliative treatment, that is, treatment designed to achieve at least partial relief of symptoms insofar as possible, but not curing the disorder or impairmant completely.

The term "preventing" as used herein is meant to include both avoiding the onset of the disorder or impairment thereby avoiding the occurrence of the characteristic symptoms associated with the disorder or impairment.

The term "ameliorating" as used herein refers to any indicia of success in the treatment or amelioration of a disorder or impairment, condition, or symptom associated with cognitive impairment, including any objective or subjective parameter such as spatial memory; diminishing of symptoms or making the disorder, impairment, condition, or symptom more tolerable to the subject; decreasing the frequency or duration of the disorder, impairment, condition, or symptom; and improving a subject's physical or mental well-being.

The term "cognitive impairment" as used herein refers to diminished or reduced cognitive function, and includes "learning disability", which term refers to a permanent, lifelong condition, usually present from birth or that develops before the age of 18, that results in subjects having impaired skills in areas such as cognition, language, motor and social abilities. Subjects suffering from cognitive impairment generally exhibit a low-average IQ, problems with visual spatial skills, memory, language, executive functioning, and attention.
The term “subject” as used herein refers to a mammal, which may be a human or a non-human mammal such as but not limited to a dog, a cat, a non-human primate such as a monkey or ape, a rabbit, a rat, a mouse, or a pig. Preferably, the subject is a human. The term preferably refers to a human child or adolescent human of which cognitive abilities are under development.

The terms "administering" and “administration” as used herein are intended to include various routes of administration, including oral, sublingual, pulmonary, nasal, intramuscular, intradermal, subcutaneous, rectal, intraperitoneal and intravenous routes, which allow for the active compound subject of the present invention to perform its intended function of treating and/or preventing the occurrence or recurrence of the disorder or impairment.

The term “therapeutically effective amount” as used herein refers to a non-toxic amount of the active ingredient sufficient to provide the desired therapeutic effect, in casu, the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent, or avoiding the onset thereby avoiding the occurrence of the characteristic symptoms associated with cognitive impairment in a subject, diminishing of symptoms or making the cognitive impairment more tolerable to the subject, decreasing the frequency or duration or improving the physical or mental well-being of a subject suffering from disorders associated with (development of) cognitive impairment or the development of that disorder. The exact amount will vary from subject to subject depending on the age of the subject, their general health, the severity of the disorder being treated and the mode of administration. It is therefore not possible to specify an exact “therapeutically effective amount”, however one skilled in the art would be capable of determining a “therapeutically effective amount” by routine trial and experimentation.

The term “hyperpolarization-activated cyclic nucleotide-gated (HCN) channel” as used herein refers to the proteins that serve as ion channels across
the plasma membrane of heart and brain cells. HCN channels, sometimes referred to as "pacemaker channels", underlie the pacemaker currents in neurons and cardiac cells designated as $I_h$ and $I_f$, respectively, and help to generate rhythmic activity within groups of heart and brain cells. HCN channels are encoded by four genes (HCN1-4). All of these four HCN isoforms are expressed in the mammalian brain, however HCN1 and HCN2 show the highest expression in the hippocampus. The biophysical properties of $I_h$ vary among different cells, reflecting the diverse functions of these currents, including determination of the resting membrane potential, dendritic integration, pacemaker activity, and modulation of synaptic transmission. HCN channels are activated at negative membrane potentials and specifically upon repolarization following action potential firing resulting in a depolarizing current influencing the threshold for subsequent action potential generation. Consequently, HCN channels and $I_h/I_f$ play a critical role in regulating excitability and rhythmic activity in excitable cells. In particular, as referred to herein, HCN channels are family of voltage-gated ion channels that are activated upon membrane hyperpolarization and mediate a non-selective cationic current named $I_h$. Subtle modifications in the physiology of HCN channels can provide significant changes in synaptic integration and neuronal excitability.

The term “HCN channel-mediated non-selective cationic current ($I_h$)” as used herein refers to the neuron-specific current which can for instance be measured by whole-cell patch clamp recording of neurons.

The term “agonist” as used herein refers to a molecule having the ability to initiate or enhance a biological function of a target polypeptide. Accordingly, the term "agonist" is defined in the context of the biological role of the target polypeptide. While preferred agonists herein specifically interact with (e.g. bind to) the target, molecules that initiate or enhance a biological activity of the target polypeptide by interacting with other members of the signal transduction pathway of which the target polypeptide is a member are
also specifically included within this definition. Agonists, as defined herein, without limitation, include antibodies and immunoglobulin variants, peptides, peptidomimetics, non-peptide small molecules, antisense molecules, and oligonucleotide decoys.

The term “HCN agonist” includes each and any compound that stimulates or activates the HCN channel current. Stimulation of HCN channel activation may therefore be measured by determining the HCN current (Ih) in a neuron or other HCN channel-comprising cell, preferably in an inhibitory neuron, and determining that the HCN current is increased as a result of the administration of the agonist. One suitable, and preferred HCN agonist is Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine). Other suitable HCN agonists are gamma-amino butyric acid (GABA) analogs, preferably 3-alkylated GABA analogs such as Gabapentin and Pregabalin; optionally substituted benzazepines such as the D(1) dopamine receptor agonist SKF83959 as may be obtained from the National Institute of Mental Health Chemical Synthesis Program (Bethesda, MD); polypeptide hormones, in particular peptide neurotransmitters such as Corticotropin Releasing Hormone [CRH]; and 5-hydroxytryptamine (serotonin) receptor 4 agonists (or 5-HT4 receptor agonists) such as BIMU-8 [4-ethylphenylamino-1,2-dimethyl-6-((endo-N-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dehydro-2-oxo-3-(prop-2-yl)-IChbenzimidazole-1-carboxamide)], Cisapride, CJ-033,466, ML-10302, Mosapride, Prucalopride, Renzapride, RS-67506, RS-67333, SL65.0155, Tegaserod, Zacopride and other (substituted) benzamides, preferably Prucalopride. Without wishing to be bound by theory, it is believed that over-inhibition of excitatory neurons is associated with learning disabilities, and associated cognitive or psychiatric impairment or developmental intellectual disability (DID) as referred to herein. This over-inhibition is the result of hyperactivity of inhibitory neurons. Hence, by inhibiting the functioning of inhibitory neurons through administration of a HCN channel agonist, i.e., through stimulation of the Ih current in those inhibitory neurons, the over-
inhibition of excitatory neurons is dampened and learning abilities are improved. Thus, a "HCN agonist" in aspects of the present invention includes each and any compound that stimulates, increases or activates the HCN channel current in an inhibitory neuron resulting in a reduction of the inhibition of excitatory neurons in the brain by these inhibitory neurons. Such compound may include phenyltriazines, GABA analogs, (substituted) benzazepines, peptide neurotransmitters and (substituted) benzamides. Preferably the HCN agonist is selected from phenyltriazines, GABA analogs, (substituted) benzazepines, and (substituted) benzamides, more preferably from phenyltriazines, GABA analogs, and (substituted) benzamides, and even more preferably from phenyltriazines and (substituted) benzamides.

The term “Neurofibromatosis type I (NF1)” as used herein refers to a human genetic disorder formerly known as von Recklinghausen disease caused by a mutation of the NF1 gene on the long arm of chromosome 17 which encodes a protein known as neurofibromin that plays a role in cell signaling. The Neurofibromin 1 gene is a negative regulator of the Ras oncogene signal transduction pathway. It stimulates the GTPase activity of Ras. The mutant gene is transmitted with an autosomal dominant pattern of inheritance, but up to 50% of NF-1 cases arise due to spontaneous mutation. The incidence of NF-1 is about 1 in 3500 live births.

The term “dosage” as used herein generally refers to a "unit dosage form" as a physically discrete unit suitable as unitary dosage for human and animal subjects, each unit containing a predetermined quantity of compounds subject of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutical acceptable diluent, carrier or vehicle. The specifications for the unit dosage forms for use in the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.
The term "cognitive training session" as used herein refers to repetitive exercise of higher-level cognitive functions that are primarily mediated by the frontal lobes. These functions include insight, awareness, judgment, planning, organization, problem solving, multi-tasking and working memory.

The term "pharmaceutically acceptable carrier" as used herein refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, for reconstitution into sterile topical ointments and/or creams. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, methanol, isopropanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil, light mineral oil, cottonseed oil, castor oil, and the like) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

The term "lamotrigine" as used herein refers to the anticonvulsant drug used in the treatment of epilepsy and bipolar disorder, and which is further used to treat partial seizures, primary and secondary tonic-clonic seizures, and seizures associated with Lennox-Gastaut syndrome. Lamotrigine is chemically 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine. It has an empirical formula of C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>Cl<sub>2</sub>, a molecular weight of 256.09 and the structural formula shown below.
The preparation of lamotrigine is described in U.S. Pat. No. 4,602,017, which is incorporated in its entirety herein by reference for all purposes. Lamotrigine is very slightly soluble in water (~0.17 mg/ml at 25° C.; ~0.57 mg/ml at 37° C.), but is significantly more soluble in 0.01N HCl (~3 mg/ml at 25° C.) and 0.1N HCl (~4 mg/ml at 25° C.).

The term “analogue” as used herein refers to a chemical compound that is structurally similar to a parent compound, but differs slightly in composition (e.g., one atom or functional group is different, added, or removed). The analogue may or may not have different chemical or physical properties than the original compound and may or may not have improved biological and/or chemical activity. For example, the analogue may be more hydrophilic or it may have altered reactivity as compared to the parent compound. The analogue may mimic the chemical and/or biologically activity of the parent compound (i.e., it may have similar or identical activity), or, in some cases, may have increased or decreased activity. The analogue may be a naturally or non-naturally occurring (e.g., recombinant) variant of the original compound. An example of an analogue is a mutein (i.e., a protein analogue in which at least one amino acid is deleted, added, or substituted with another amino acid). Other types of analogues include isomers (enantiomers, diasteromers, and the like) and other types of chiral variants of a compound, as well as structural isomers. The analogue may be a branched or cyclic variant of a linear compound. For example, a linear compound may have an analogue that is branched or otherwise substituted to impart certain desirable properties (e.g., improve hydrophilicity or bioavailability).
The term "derivative" as used herein refers to a chemically or biologically modified version of a chemical compound that is structurally similar to a parent compound and (actually or theoretically) derivable from that parent compound. A "derivative" differs from an "analogue" in that a parent compound may be the starting material to generate a "derivative," whereas the parent compound may not necessarily be used as the starting material to generate an "analogue." A derivative may or may not have different chemical or physical properties of the parent compound. For example, the derivative may be more hydrophilic or it may have altered reactivity as compared to the parent compound. Derivatization (i.e., modification) may involve substitution of one or more moieties within the molecule (e.g., a change in functional group). For example, a hydrogen may be substituted with a halogen, such as fluorine or chlorine, or a hydroxyl group (—OH) may be replaced with a carboxylic acid moiety (—COOH). The term "derivative" also includes conjugates, and prodrugs of a parent compound (i.e., chemically modified derivatives which can be converted into the original compound under physiological conditions). For example, the prodrug may be an inactive form of an active agent. Under physiological conditions, the prodrug may be converted into the active form of the compound. Prodrugs may be formed, for example, by replacing one or two hydrogen atoms on nitrogen atoms by an acyl group (acyl prodrugs) or a carbamate group (carbamate prodrugs). More detailed information relating to prodrugs is found, for example, in Fleisher et al., Advanced Drug Delivery Reviews 19 (1996) 115; Design of Prodrugs, H. Bundgaard (ed.), Elsevier, 1985; or H. Bundgaard, Drugs of the Future 16 (1991) 443. The term "derivative" is also used to describe all solvates, for example hydrates or adducts (e.g., adducts with alcohols), active metabolites, and salts of the parent compound. The type of salt that may be prepared depends on the nature of the moieties within the compound. For example, acidic groups, for example carboxylic acid groups, can form, for example, alkali metal salts or alkaline earth metal salts (e.g., sodium salts, potassium salts,
magnesium salts and calcium salts, and also salts with physiologically tolerable quaternary ammonium ions and acid addition salts with ammonia and physiologically tolerable organic amines such as, for example, triethylamine, ethanolamine or tris-(2-hydroxyethyl)amine). Basic groups can form acid addition salts, for example with inorganic acids such as hydrochloric acid, sulfuric acid or phosphoric acid, or with organic carboxylic acids and sulfonic acids such as acetic acid, citric acid, benzoic acid, maleic acid, fumaric acid, tartaric acid, methanesulfonic acid or p-toluenesulfonic acid. Compounds which simultaneously contain a basic group and an acidic group, for example a carboxyl group in addition to basic nitrogen atoms, can be present as zwitterions. Salts can be obtained by customary methods known to those skilled in the art, for example by combining a compound with an inorganic or organic acid or base in a solvent or diluent, or from other salts by cation exchange or anion exchange.

The term “pharmaceutically acceptable salts” as used herein includes, but is not limited to, those formed from: acetic, ascorbic, aspartic, benzoic, benzenesulfonic, citric, cinnamic, ethanesulfonic, fumaric, glutamic, glutaric, gluconic, hydrochloric, hydrobromic, lactic, maleic, malic, methanesulfonic, naphthoic, hydroxynaphthoic, naphthalenesulfonic, naphthalenedisulfonic, naphthaleneacrylic, oleic, oxalic, oxaloacetic, phosphoric, pyruvic, p-toluenesulfonic, tartaric, trifluoroacetic, triphenylacetic, tricarballylic, salicylic, sulfuric, sufamic, sulfanilic and succinic acid.

The term “up-regulated” as used herein refers to an increase in amount, volume, frequency, amplitude or any other parameter that is upregulated wherein that parameter is measured relative to a wild-type or non-disease control reference, wherein the increase is at least an increase by 5%, preferably 10%, most preferably 50-100% of the parameter value in the reference.

The term “parvalbumin immune-positive (PV+) hippocampal inhibitory neuron (interneuron)” as used herein refers to a specific type of
GABAergic inhibitory neuron. Most inhibitory neurons of the mammalian forebrain use γ-aminobutyric acid (GABA) as a neurotransmitter. GABA is released presynaptically in response to action potentials and diffuses across the synaptic cleft where it binds to postsynaptic receptors directly coupled to ion channels permeable to anions. Activation of these receptor-linked ion channels hyperpolarizes, or inhibits, postsynaptic neurons. Whether a hippocampal inhibitory neuron is of a PV⁺ subtype can be determined by methods well known in the art, for instance as described in Wulff et al. 2009. PNAS 106(9), pp. 3561-3566 (and in particular in the section Immunohistochemistry of the Supporting Information).

The term “Nf1⁺/+” mouse” as used herein refers to a mouse in which one of the two NF1 alleles has been mutated in such a way that this allele can not be used to make the full-length NF1 protein. Hence, this mouse has only one functional NF1 gene, which resembles the genotype of most NF1 patients. Nf1⁺/− mice are publicly available through The Jackson Laboratory (Bar Harbor, ME, USA), as JAX® Mice Database Stock Number 008192.

The term “Nf1¹⁶⁺/⁺⁹⁺⁺/⁹⁺⁺” mouse” as used herein refers to a mouse in which both NF1 alleles have been mutated in such a way that the exon 9a is not incorporated in the NF1 protein. The generation of NF1-exon 9a mutant mice is described in the Examples below.

Preferred embodiments

The invention relates inter alia to a method for the treatment or prophylaxis of a condition associated with cognitive impairment in a subject in need thereof comprising the administration to the subject of a therapeutically effective amount of an HCN channel agonist, as defined above. The condition associated with cognitive impairment may be low-average IQ, or any one of problems with visuospatial skills, memory, language, executive functioning, or attention.
An HCN channel agonist is useful as therapeutic agent in the treatment or prevention of conditions associated with cognitive impairment as defined above. An HCN channel agonist may suitably be administered to a subject (for example a human) in the form of a pharmaceutical composition.

Pharmaceutical compositions include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), inhalation (including use of metered dose pressurized aerosols, nebulizers or insufflators), rectal and topical (including dermal, buccal, sublingual and intraocular) administration.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing an HCN channel agonist as defined herein into association with a carrier, which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the HCN channel agonist with a liquid carrier or finely divided solid carrier, or both and then, if necessary, shaping the product into the desired composition.

Generally, an effective dosage of an HCN channel agonist present in pharmaceutical and other compositions of the present invention is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours, or about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range of about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours, or about
5.0mg to about 15mg per kg body weight per 24 hours. Preferably, a dosage is about 5-30 mg/kg body weight preferably per 24 hours, more preferably less than 10 mg/kg.

Compositions suitable for buccal (sublingual) administration include lozenges comprising an HCN channel agonist in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising an HCN channel agonist in an inert base such as gelatine and glycerin or sucrose and acacia.

Compositions comprising an HCN channel agonist suitable for oral administration may be presented as discrete units such as gelatine or HPMC capsules, cachets or tablets, each containing a predetermined amount of an HCN channel agonist, as a powder, granules, as a solution or a suspension in an aqueous liquid or a non-aqueous liquid, or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. An HCN channel agonist may also be present in a paste.

When the compositions comprising an HCN channel agonist are formulated as capsules, the an HCN channel agonist may be formulated with one or more pharmaceutically acceptable carriers such as starch, lactose, microcrystalline cellulose, silicon dioxide and/or a cyclic oligosaccharide such as cyclodextrin. Additional ingredients may include lubricants such as magnesium stearate and/or calcium stearate. Suitable cyclodextrins include oc-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, 2- hydroxyethyl" -cyclodextrin, 2- hydroxypropyl-cyclodextrin, 3- hydroxypropyl" -cyclodextrin and tri-methyl- cyclodextrin. The cyclodextrin may be hydroxypropyl- -cyclodextrin. Suitable derivatives of cyclodextrins include Captisol® a sulfobutyl ether derivative of cyclodextrin and analogues thereof as described in US patent No. 5,134,127.

Tablets may be prepared by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine an HCN channel agonist in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant (for example magnesium stearate or calcium stearate), inert diluent
or a surface active/dispersing agent. Moulded tablets may be made by
moulding a mixture of the powdered an HCN channel agonist moistened with
an inert liquid diluent, in a suitable machine. The tablets may optionally be
coated, for example, with an enteric coating and may be formulated so as to
provide slow or controlled release of an HCN channel agonist therein.

Compositions for parenteral administration include aqueous and
non-aqueous sterile injectable solutions which may contain anti-oxidants,
buffers, bacteriostats and solutes which render the formulation isotonic with
the blood of the intended recipient, and which may include suspending agents
and thickening agents. A parenteral composition may comprise a cyclic
oligosaccharide such as hydroxypropyl-cyclodextrin. The compositions may be
presented in unit-dose or multi-dose containers, for example sealed ampoules
and vials, and may be stored in a freeze-dried (lyophilised) condition requiring
only the addition of the sterile liquid carrier, for example saline or water-for-

Compositions suitable for transdermal administration may be
presented as discrete patches adapted to remain in intimate contact with the
epidermis of the recipient for a prolonged period of time. Such patches suitably
comprise an HCN channel agonist as an optionally buffered aqueous solution
of, for example, 0.1 M to 0.2 M concentration with respect to the compound.

Compositions suitable for transdermal administration may also be
delivered by iontophoresis, and typically take the form of an optionally
buffered aqueous solution of the active compound. Suitable compositions may
comprise citrate or Bis/Tris buffer (pH 6) or ethanol/water and contain from 0.1
M to 0.2 M of an HCN channel agonist.

Spray compositions for topical delivery to the lung by inhalation
may, for example be formulated as aqueous solutions or suspensions or as
aerosols, suspensions or solutions delivered from pressurized packs, such as a
metered dose inhaler, with the use of a suitable liquefied propellant. Suitable
propellants include a fluorocarbon or a hydrogen-containing chlorofluorocarbon
or mixtures thereof, particularly hydrofluoroalkanes, e.g.
dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane,
especially 1,1,1,2-tetrafluoroethane, 1,1,2,2,3,3,3-heptafluoro-n-propane or a
mixture thereof. Carbon dioxide or other suitable gas may also be used as
propellant. The aerosol composition may be excipient free or may optionally
contain additional composition excipients well known in the art, such as
surfactants e.g. oleic acid or lecithin and cosolvents e.g. ethanol. Pressurized
compositions will generally be retained in a canister (e.g. an aluminum
canister) closed with a valve (e.g. a metering valve) and fitted into an actuator
provided with a mouthpiece.

Medicaments for administration by inhalation desirably have a
controlled particle size. The optimum particle size for inhalation into the
bronchial system is usually 1-10 \( \mu \text{m} \), preferably 2-5 \( \mu \text{m} \). Particles having a size
above 20 \( \mu \text{m} \) are generally too large when inhaled to reach the small airways.

When the excipient is lactose it will typically be present as milled lactose,
wherein not more than 85\% of lactose particles will have a MMD of 60-90 \( \mu \text{m} \)
and not less than 15\% will have a MMD of less than 15 \( \mu \text{m} \). Compositions for
rectal administration may be presented as a suppository with carriers such as
cocoa butter or polyethylene glycol, or as an enema wherein the carrier is an
isotonic liquid such as saline. Additional components of the compositions may
include a cyclic oligosaccharide, for example, a cyclodextrin, as described
above, such as hydroxypropyl-cyclodextrin, one or more surfactants, buffer
salts or acid or alkali to adjust the pH, isotonicity adjusting agents and/or anti-
oxidants.

Compositions suitable for topical administration to the skin
preferably take the form of an ointment, cream, lotion, paste, gel, spray,
aerosol, or oil. Carriers which may be used include Vaseline, lanoline,
polyethylene glycols, alcohols, and combination of two or more thereof. An
HCN channel agonist is generally present at a concentration of from 0.1\% to
20% w/w, or from 0.5% to 5% w/w. Examples of such compositions include cosmetic skin creams.

The composition may also be administered or delivered to target cells in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances and are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Specific examples of liposomes that may be used to administer or deliver a compound formula (I) include synthetic cholesterol, 1,2-distearoyl-Sft-glycero-3-phosphocholine, 3-N-[(methoxy poly(ethylene glycol)2000)carbamoyl]-1,2-dimyrestyloxy-propylamine (PEG-cDMA) and 1,2-di-o-octadeceny1-3-(N,N-dimethyl)aminopropane (DODMA).

The compositions may also be administered in the form of microparticles. Biodegradable microparticles formed from polylactide (PLA), polylactide-co-glycolide (PLGA), and ε-caprolactone have been extensively used as drug carriers to increase plasma half life and thereby prolong efficacy (R. Kumar, M., 2000, J Pharm Pharmacut Sci. 3(2) 234-258).

The compositions may incorporate a controlled release matrix that is for instance composed of sucrose acetate isobutyrate (SAIB) and organic solvent or organic solvent mixtures. Polymer additives may be added to the vehicle as a release modifier to further increase the viscosity and slow down the release rate.

The invention will now be exemplified by the following non-limiting Examples.
EXAMPLES

Materials and Methods to the Examples

5 Generation of NF1-exon 9a mutant mice.

NF1 mutant ES cells were generated as follows: A 1.5 Kb intronic DNA fragment was obtained by PCR using primers designed within exon 9 and exon 9a of the NF1 gene. This fragment was used to screen a C57Bl6 genomic DNA library. A 6 Kb fragment encoding exon 9 and exon 9a was obtained. A PGK neomycin cassette flanked by LoxP sites was inserted in the SacI site, which is approximately 500 bp downstream of the exon 9a (FIGURE 1a). To make the neuronal specific deletion of NF1 9a (NF1<sup>9a−/9a−</sup>), a stop codon as well as a frameshift mutation was introduced within exon 9a. After transfection of R1 embryonic stem cells (derived from 129/Sv substrain (a Nagy et al. 1993) and selection with G418, targeted clones (approximately 4%) were identified by Southern blot analysis. After checking the presence of the mutations by PCR/restriction analysis and sequencing, the PGK neo cassette was removed by a secondary transient transfection with pBS185 (Gibco) encoding the Cre recombinase. F1 heterozygous mice were obtained by injection of the ES cells into blastocysts of C57BL/6J mice, to obtain chimeric mice. Mutants were back-crossed 13 times into C57BL/6JOlaHsd (Harlan) and maintained in C57BL/6JOlaHsd (Harlan). Since NF1 expression and phenotype is background dependent, and we wanted to compare our data with published literature on NF1<sup>+/−</sup> mice, we obtained the experimental mice by crossing NF1<sup>9a−/9a−</sup> mice (in B6 background) with 129T2/SvEmsJ (Jackson) and subsequently crossed the obtained F1 mutant mice with each other to get homozygous NF1<sup>9a−/9a−</sup> mutants and wild-type littermates in the hybrid B6/129T2 background. Homozygous NF1<sup>9a−/9a−</sup> mice are born at the expected Mendelian frequency, and were undistinguishable from wild-type mice upon examination by an experienced observer. The experimenter was blind to the genotype. Mice were housed on a 12 hours/ light/dark cycle with food and
water available *ad libitum*. All animal procedures were approved by a Dutch
Ethical Committee (DEC) for animal experiments.

**Behavioral experiments with Lamotrigine**

All mice were between 8 and 15 weeks of age at the start of the
behavioural experiments. The animals were housed in group-cages (2 to 4
same-sex animals), on a 12h light/dark cycle with light phases between 700h
and 1900h. Experiments were executed between 1300h and 1730h. Mice were
fed standard laboratory food ad libitum. All animals were handled for 3 days
before the first injection and were given intraperitoneal injection of vehicle or
Lamotrigine (25 mg/kg) for 7 days before the first experiment and two-three
hours before the first trial of each day during behavioural experiments. The
experimenter executing the behavioural tasks was masked to the genotype and
treatment of the mice.

For motor learning, *Nf1*+/− mice were tested on the accelerating
Rotarod (Ugo Basile, Comerio Varese, Italy) as described previously (van der
Vaart et al., 2011, Genes Brain Behav 10, 404–409).

To test spatial memory we used a standard watermaze task. Prior to
the test, mice were handled extensively (2 min/day; 5 days). After handling
period, mice were trained in a circular water tank (120 cm in diameter, 40 cm
high) to find an escape platform (11 cm of diameter) 1 cm below the surface of
the water. The pool contained water (26 °C) made opaque by the addition of an
inert and nontoxic product. Both the pool and the surrounding distal cues were
kept fixed during all experiments. We use dimmed lighting, and mouse-
tracking is performed using SMART version 2.0 (Panlab, Barcelona, Spain).
Mice were given 4 trials per day, with 1 hour inter trial interval for 5
consecutive days. A trial consists of 2 training sessions where the mouse was
first placed on the platform for 30 sec. Then it was placed in the water at a
pseudo-random start position and it was given a maximum of 60 seconds to
find the platform. If the mouse did not find the platform within 60 seconds, it
was placed back on the platform. After 30 seconds on the platform, this training procedure was repeated. The platform position remained at the same position during all trials. Probe trials were given to test spatial learning. In such probe trials, mice were placed on the platform for 30 seconds, after which the platform was removed from the pool at the opposite side of the previous platform position. The mice were then allowed to search for the platform for 60 seconds.

All experiments were approved by the Dutch Ethical Committee and were in accordance with the institutional animal care and use committee guidelines.

**Electrophysiology**

Hippocampal slices were prepared from the brain of 3- to 4 week-old and adult mice using standard techniques. Briefly, animals were decapitated and brains quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 3 KCl, 1.3 CaCl₂, 2.5 MgSO₄, 1.25 NaH₂PO₄, 10 glucose, 26 NaHCO₃, and 212.3 sucrose bubbled with 95% O₂ and 5% CO₂ (pH 7.3–7.4). Transverse hippocampal slices (300 μm) were cut and stored in a holding chamber containing control ACSF (in mM): 126 NaCl, 3 KCl, 2 MgSO₄, 2 CaCl₂, 10 glucose, 1.25 NaH₂PO₄ and 26 NaHCO₃ bubbled with 95% O₂ and 5% CO₂ (pH 7.3). Slices were recovered at room temperature for at least 1 h and then individual slices were transferred to the submerged recording chamber perfused with control ACSF solution continuously bubbled with 95% O₂ and 5% CO₂ at 30–33°C.

Extracellular recording of field excitatory postsynaptic potentials (fEPSPs) were made in CA1 striatum radiatum with a platinum/iridium electrodes (FHC, Bowdoinham, ME). For all extracellular recording, the area CA3 was removed before placing the slice in recording chamber. A bipolar twisted platinum/iridium was used to stimulate Schaffer collateral/commissural afferents orthodromically. At the beginning of each
experiment, the strength of the stimulus pulse (100-μsec duration) was adjusted to obtain a response equal to one-third of the maximal fEPSP, and was then fixed at this level. At least 10 min of stable baseline recordings were obtained prior to the delivery of drugs and/or long-term potentiation (LTP) protocols. LTP was induced using different LTP protocols including: 10 Hz (100 pulse for 10 s) and 100 Hz (1s) and theta burst stimulation (TBS) protocols. TBS protocol consisting of two or four trains at 5 Hz composed each of four pulses at 100 Hz.

Whole-cell patch-clamp recording were performed from hippocampal CA1 pyramidal neurons visualized with an Olympus BX51W1 microscope using infrared video microscopy and differential interference contrast (DIC) optics. Patch electrodes were pulled from borosilicate glass capillaries and had a resistance of 3-5 MΩ when filled with one of two intracellular solutions. For IPSCs recording internal solution contained (in mM): CsCl 125, HEPES 10, EGTA 0.2, MgCl₂ 2, NaCl 1, MgATP 2, Na₂GTP 0.5 (pH 7.3 with CsOH). For EPSCs and EPCPs (in mM: K-gluconate 140, HEPES 10, EGTA 0.5, KCl 1, MgATP 4, Na₂GTP 0.4, K₂-phosphocreatine 4 (pH 7.3 with KOH). Recordings were made with a patch-clamp amplifier (Multiclamp 700B; Axon Instruments, Foster City, CA, USA). Signals were low-pass filtered at 3 or 10 KHz and digitized at 10 KHz. Series resistance was constantly monitored, and the cells were rejected if the resistance changed by >20%. No series resistance compensation was used. In voltage-clamp recordings, the holding potential was set to -70 mV. Miniature inhibitory post synaptic currents (mIPSCs) were recorded in the presence of 1 μM TTX, 10 μM 6-cyano-7- nitroquinoxaline-2,3-dione (CNQX) and 20 μM D,L-2-amino-5-phosphopentanoic acid (D,L-AP5). Miniature excitatory post synaptic currents (mEPSCs) were recorded in the presence of 1 μM TTX and 100 μM picROTOXIN.

Synaptic responses were evoked using twisted platinum/iridium electrodes. Stimulating electrode was placed near the stratum pyramidale (~150 μm from the recording site) to stimulate interneurons and their axon
arbors. Paired-pulse stimulation of the local inhibitory interneurons at an interpulse interval of 50 ms was used to measure paired pulse ratio (PPR), calculated as the ratio of mean IPSC2 amplitude/mean IPSC1 amplitude.

All drugs were made from stock solutions in either water or DMSO (the final concentration of DMSO, ≤0.1%) accordingly. Picrotoxin and D,L-AP5 were obtained from Tocris Cookson Ltd. CNQX, U0126 and TTX were obtained from Sigma.

Data Analysis

Miniature synaptic currents (mIPSCs and mEPSCs) data were analyzed with Mini Analysis (Synaptosoft Inc., Decatur, GA). All events were detected with a criterion of a threshold >3× root-mean-square (RMS) of baseline noise. The detected currents manually inspected to exclude false events. Evoked potentials were analyzed offline using pClamp10 (Axon Instruments). Changes in synaptic strength were quantified for statistical comparisons by normalizing and averaging fEPSP slopes during the last 10 min of experiments relative to baseline. All data are reported as means ± s.e.m. Statistical comparisons were made using ANOVA, Student’s t test and the Kolmogorov–Smirnov test.

HCN measurements

HCN channels slowly activate in response to hyperpolarization and generate an inward depolarizing current, termed I_h. To examine the properties of I_h in PV cells, whole-cell voltage-clamp experiments were performed. In these experiments, the soma was clamped at -50 mV and then stepped to -120 mV in 10 mV increments and followed by a final step to -120 mV to obtain tail currents. Step hyperpolarizations from a holding potential of -50 mV resulted in slowly activation inward currents, followed at the end of voltage steps by tail currents. I_h was isolated by subtracting currents traces before and after application of ZD7288 and these subtracted currents were used for further
analysis. The current-voltage (I-V) relation of I_h was obtained by plotting steady-state currents at end of each voltage step as a function of step potential. The voltage-dependent activation of I_h was estimated from tail currents generated by a step to -120 mV. Tail currents were normalized, plotted as a function of the preceding hyperpolarization step voltage, and fitted with Boltzmann function. Based on sigmoidal fits to activation values, the half-activation potential (V_{1/2}) was determined.

To determine the time constant(s) of I_h activation and inactivation, a mono-exponential function was fitted to the current traces for I_h activation and inactivation. Activation and deactivation time constants were averaged and plotted.

Example 1. Nf1^{9a+/9a-} mice have reduced HCN channel function

To explore the function of NF1-exon 9a isoform, a proteomics screen was performed on neurofibromin-associated proteins by co-immunoprecipitation of NF1 from wild-type and Nf1^{9a+/9a-} brain extracts. Hyperpolarization-activated cation (HCN) was co-immunoprecipitated with NF1. To confirm the interaction between NF1 and HCN, we performed co-immunoprecipitation-immunoblotting assays on brain extracts. Western blot analysis of the co-immunoprecipitation confirmed that HCN1 co-immunoprecipitated with a NF1 antibody (FIGURE 1).

Having shown the putative interaction of NF1 with HCN protein and selective increase in GABA-mediated inhibition in Nf1^{9a+/9a-} mice, we sought to measure HCN current from interneuron in hippocampal CA1 region to test whether HCN channel function is indeed affected. In hippocampus, HCN channels are expressed in both pyramidal neurons and interneurons. Among the various interneurons that inhibit pyramidal cells, those that express parvalbumin (PV) have attracted greatest attraction because they express HCN1 in both somato-dendritic and axon terminals. Furthermore, majority of these interneurons innervate the perisomatic regions of pyramidal
neurons, an optimal location to control the number of active pyramidal cells and their action potential frequency by feedforward and feedback. In addition these inhibitory cells initiated the majority of inhibitory events in their target cells. Therefore, we recorded interneurons with soma located in the stratum pyramidale or at the border of the stratum pyramidale and oriens of the CA1 region. From interneurons only those cells were included in this study which could be identified as fast-spiking parvalbumin expressing interneurons (PV cells) based on their action potential phenotype including brief action potentials, pronounced afterhyperpolarization (AHP) and non-adapting high-frequency firing in response to depolarizing current pulse injection, and immune-reactivity for PV as determined by post hoc revelation of the biocytin filled neurons (see Materials and Methods).

HCN channels slowly activate in response to hyperpolarization and generate an inward depolarizing current, termed I_h. To examine the properties of I_h in PV cells, whole-cell voltage-clamp experiments were performed. In these experiments, the soma was clamped at -50 mV and then stepped to -120 mV in 10 mV increments and followed by a final step to -120 mV to obtain tail currents. TTX (1 μM) and Ba^{2+} (500 μM) were bath applied to block Na^+, Kir, and K^+-selective leak channels, thereby preventing spike generation, increasing membrane resistance, and improving voltage control of dendritic regions. A representative recording of I_h in a PV cell is shown in figure 4A. Step hyperpolarizations from a holding potential of -50 mV resulted in slowly activation inward currents, followed at the end of voltage steps by tail currents. These currents were blocked in the presence of ZD7288 (30 μM) indicating that these cells express a hyperpolarization-activated conductance with pharmacological properties characteristic of I_h. I_h was isolated by subtracting currents traces before and after application of ZD7288 (FIGURE 2A) and these subtracted currents were used for further analysis. First, we determined the current-voltage (I-V) relation of I_h obtained by plotting steady-state currents at end of each voltage step. We found that I_h currents recorded
in PV cells from NfI8α+/8α* mice was consistently smaller at potentials negative to -80 mV (FIGURE 2B). Next, the voltage-dependent activation of I_h was measured based on the normalized tail currents and fitting the current-voltage relationship with a Boltzmann function. We found that the I_h activation was shifted to more hyperpolarized potentials in PV cells from NfI8α+/8α* mice (FIGURE 2C). Based on sigmoidal fits to activation values, the half-activation potential (V_{1/2}) for I_h in PV cells from NfI8α+/8α* mice was -88.4±1.9 mV (n=10), compared to -81.2± 1.9 mV (n=7, P=0.012) in control cells. A shift in the voltage dependence of I_h activation toward negative potentials could in principle contributes to or underlies a decrease in I_h amplitude. Finally, we analyzed the kinetics of I_h by fitting current traces with a mono exponential function. The rate of current activation was voltage-dependent such that the activation time constants extracted from these functions decreased with increasing hyperpolarization. Current deactivation was also voltage-dependent and time constants decreased with increasing depolarization. We found no significant difference in the activation and deactivation kinetics of I_h between PV cells from NfI8α+/8α* mice and wild-type controls (data not shown).

To see if there is any parallel change in I_h in CA1 pyramidal neurons, we used the same voltage-clamp protocol for recording I_h in pyramidal neurons as shown in a representative recording in FIGURE 4E. We found no significant difference neither in the amplitude nor in the voltage-dependence of I_h between neurons from wild-type and mutants (FIGURE 2F,G). The V_{1/2} was -3.9±1.1 mV (n=9) in wild-type and -85.9±1.8 mV (n=10, P=0.35) in NfI8α+/8α* pyramidal neurons. Again, pyramidal neurons from two groups did not significantly differ in activation and deactivation time constants. The similarities of both voltage-dependent activation and time constants demonstrate that I_h is equivalent in pyramidal neurons of NfI8α+/8α* and wild-type mice.
Example 2. CA1 interneurons exhibit altered intrinsic membrane properties

The HCN channel down-regulation seen here would be predicted to cause neuronal hyperexcitability, as shown by a number of previous studies (Magee, 1999, Nat Neurosci 2, 508–514; Poolos et al., 2002, Nat Neurosci 5, 767–774; Nolan et al., 2004, Cell 119, 719–732; Shah et al., 2004, Neuron 44, 495–508). We tested the effects of Ih down-regulation on neuronal excitability by determining whether resting membrane potential and input resistance, two parameters sensitive to the amount of Ih active at rest, were affected in our mutants. Current-clamp recordings from PV cells revealed that in contrast to PV cells from wild-type mice, those from Nf1<sup>9a<sup>*<sub>/*</sub> mice had significantly more negative resting membrane potentials (-64.1±0.7 mV, n=17 in Nf1<sup>9a<sup>*<sub>/*</sub> and -60.6±0.7 mV, n=12 in wild-type mice, p=0.003), and higher input resistance (279±19 MΩ in Nf1<sup>9a<sup>*<sub>/*</sub> , n=15 and 235±14 MΩ, n=16 in wild-type mice, p=0.04). Next, we examined the relationship between somatic current injection and action potential (AP) firing in PV cells from Nf1<sup>9a<sup>*<sub>/*</sub> mice and wild-type mice. As shown in FIGURE 2E, the depolarizing current pulse evoked a train of action potentials in PV cells. However, the input–output relationship was shifted to the left in PV cells from Nf1<sup>9a<sup>*<sub>/*</sub> mice. The number of action potentials significantly higher at most current injections in PV cells of Nf1<sup>9a<sup>*<sub>/*</sub> mice was significantly higher than in wild-type despite the fact that Vm was more hyperpolarized (FIGURE 2F). These results suggest that decreased Ih in PV cells from Nf1<sup>9a<sup>*<sub>/*</sub> mice results in increased intrinsic excitability of these cells. The increase in AP firing was not likely caused by alterations in sodium channel activation as there was no significant change in action potential threshold. Consistent with this, other action potential parameters including peak amplitude, half-width and rise time of action potential were not different between groups. As the maximal rate of rise of action potential is dependent on the Na<sup>+</sup> current, whereas the half-width is
correlated with K⁺ current, these results also indicates that Na⁺ and K⁺ channels are not affected.

We next sought to determine whether the intrinsic membrane properties of CA1 pyramidal neurons are also different in *Nf1*<sup>9a⁻/9a⁻</sup> mice. Resting potential was slightly negative in pyramidal neurons of *Nf1*<sup>9a⁻/9a⁻</sup> mice but did not reach to significant level (-68.1±0.9 mV in *Nf1*<sup>9a⁻/9a⁻</sup>, n=17 and -66.5±0.8 mV, n=23 in wild-type mice, p=0.20). There was no significant difference in input resistance between *Nf1*<sup>9a⁻/9a⁻</sup> mice and wild-type controls (*Nf1*<sup>9a⁻/9a⁻</sup>: 116±4.5 MΩ, n=18 and wild-type: 118±4.5 MΩ, n=14, p=0.83).

Under control conditions, hyperpolarizing current injections evoked a hyperpolarization with characteristic depolarizing sag reflecting the slow activation of I<sub>h</sub> (FIGURE 2E). In these cells voltage sag was observed in response to a hyperpolarizing current step. Relative sag amplitude measured as ratio of peak to steady state voltage response to a hyperpolarizing current step of -200 pA was not different between groups (1.11±0.02 in *Nf1*<sup>9a⁻/9a⁻</sup> and 1.15±0.03 MΩ, in wild-type mice, p=0.25). We also found no significant change in membrane excitability of pyramidal neurons from *Nf1*<sup>9a⁻/9a⁻</sup> mice (FIGURE 2G). Consistent with our finding in CA1 interneurons, there were no significant differences in action potential parameters including the threshold, peak amplitude, half-width and rise time between two groups.

**Example 3. Blocking HCN channels mimics the NF1 phenotype of increased spontaneous GABA release**

Increased excitability attributable to down-regulation of I<sub>h</sub> in PV cells should presumably increase the spontaneous GABA release onto CA1 pyramidal neurons. Therefore, we hypothesized that blocking I<sub>h</sub> in wild-type mice would increase inhibitory output onto pyramidal neurons and mimic the enhanced inhibition observed in *Nf1*<sup>+/−</sup> (Cui et al., 2008, supra) and *Nf1*<sup>9a⁻/9a⁻</sup> mice. Spontaneous inhibitory post-synaptic currents (sIPSCs) in CA1 pyramidal neurons were recorded from CA1 pyramidal neurons before and
after application of ZD7288 (30 μM). Supporting our hypothesis, the frequency of sIPSCs recorded in CA1 pyramidal cells of wild-type and Nfilt9α+/−/9α− mice was significantly increased after application of ZD7288, although, the ZD7288-induced increase of sIPSC frequency was less pronounced in Nfilt9α+/−/9α− mice (FIGURE 3). There was no significant effect on the sIPSC amplitude in both groups indicating that the increase in sIPSC frequency most likely resulted from increased spontaneous firing of interneurons that directly provide synaptic inhibition of CA1 pyramidal neurons (FIGURE 3). Taken together, these results show the critical role of Ih in regulating inhibitory output from PV cells.

**Example 4. Pharmacological up-regulation of HCN channels reduces the excitability of PV cells and rescues the LTP deficit in Nfilt9α+/−/9α− mice**

If reduced HCN channel dysfunction is underlying the NF1 phenotype, then drugs that enhance HCN channel function may ameliorate the NF1 deficits, and hence be used as treatment for the cognitive deficits. The antiepileptic drug lamotrigine (Lamotrigine) was reported to enhance Ih in pyramidal neurons through a positive shift in the voltage dependence of Ih activation, thus increasing the channel open probability at a given physiological membrane potential (Poolos et al., 2002, Nat Neurosci 5, 767–774). Enhancement of Ih in these cells has been shown to decrease the passive propagation of excitatory postsynaptic potentials (EPSPs) from distal dendrites to the soma (Poolos et al., 2002, supra). Given the presence of Ih in interneurons, compounds that enhance Ih could also increase this current in inhibitory interneurons. Indeed, Lamotrigine was shown that increases Ih in statum-oriens interneurons (Peng et al., 2010, Neuropsychopharmacology 35, 464–472). Therefore, we investigated whether Lamotrigine similarly increases Ih in PV cells, and if so, whether this decreases excitability of these cells in Nfilt9α+/−/9α− mice. Voltage-clamp experiments were performed in the presence of
Lamotrigine (50 \, \mu\text{M}) in PV cells with the same protocol was used in control condition. The current-voltage relation showed that the magnitude of $I_h$ was significantly increased in the presence of Lamotrigine as compared to control condition (FIGURE 4A). In addition, application of Lamotrigine produced a depolarizing shift in $I_h$ activation. $V_{1/2}$ for $I_h$ in PV cells from Nf1$^{9a+/9a^*}$ mice in the presence of Lamotrigine was -80.9±2.0 mV ($n=9$) which was significantly different from that obtained in control condition in Nf1$^{9a+/9a^*}$ mice (-88.4±1.9 mV, $P=0.02$) and reaches the wild-type level (FIGURE 4B). Application of Lamotrigine caused similar increase in $I_h$ in PV cells of wild-type mice (data not shown). This modulation of $I_h$ activation would be expected to produce significant changes in the amount of $I_h$ present at resting potential and thus influence neuronal excitability. Consistent with this idea, bath application of Lamotrigine caused a depolarization of resting membrane potential (3.5±0.5 mV, $n=12$ in Nf1$^{9a+/9a^*}$ mice and 3.0±0.3 mV, $n=9$ in wild-type mice) and a decrease in action potential firing in majority of cells in both wild-type and Nf1$^{9a+/9a^*}$ mice. The effect of increased $I_h$ on firing rate as the percentage of control showed that increasing $I_h$ by Lamotrigine decreased firing rates over the entire range of current injections (FIGURE 4C). The largest effect was found with the smallest current injections (52±8% in Nf1$^{9a+/9a^*}$ mice and 61±7% in wild-type), indicating that $I_h$ most strongly affects firing rates at membrane potentials that are relatively close to resting membrane potential.

Decreased excitability in a subset of CA1 interneurons caused by Lamotrigine administration would be expected to decrease inhibition onto pyramidal cells and normalize LTP in our mutants. To test this possibility, we examined the effect of Lamotrigine on LTP at Schaffer Collateral pathway. First, we determined whether Lamotrigine modulates basal synaptic transmission CA1 hippocampus. Following wash-in of Lamotrigine, the slope of field EPSPs showed a slight increase in both genotypes. However there was no significant difference in input-output function between groups after Lamotrigine application. To evaluate effect of Lamotrigine on LTP, after
baseline recording in the presence of Lamotrigine, stimulation intensity was adjusted in a way that field EPSPs amplitude were equal to baseline before Lamotrigine application. After stable baseline recording in the presence of Lamotrigine or vehicle, TBS-LTP was induced at CA3-CA1 synapses in both groups. Under this condition, there was no significant difference in TBS-induced LTP between \( NfI^{3a x; 3a x} \) and wild-type mice in the presence of Lamotrigine (FIGURE 4E), while LTP deficit was still evident in vehicle-treated slices from \( NfI^{3a x; 3a x} \) (FIGURE 4D). In fact, application of Lamotrigine abolished the difference in LTP between \( NfI^{3a x; 3a x} \) and wild-type mice seen in control condition. These findings suggest a critical role of \( I_h \) for regulating inhibitory output from interneurons onto CA1 pyramidal neurons and its subsequent effect on LTP at CA3-CA1 synapses.

Example 5. Characterization of \( I_h \) in CA1 neurons of \( NfI^{+/-} \) mice

The increased inhibition as observed \( NfI^{3a x; 3a x} \) mice is similar to the previously reported \( NfI^{+/-} \) mice, which have heterozygous loss of the NF1 gene (Silva et al., 1997, Nat Genet 15, 281–284; Costa et al., 2002, supra; Cui et al., 2008, supra). We therefore investigated whether the observed HCN channel phenotype and rescue with Lamotrigine as shown above for \( NfI^{3a x; 3a x} \) mice, can be extended to the \( NfI^{+/-} \) mice. \( I_h \) was recorded in the whole-cell voltage clamp configuration from PV cells using the same voltage-clamp protocol (Figure 5A). The current-voltage (I-V) relation of \( I_h \) showed smaller amplitude of \( I_h \) in the PV cells in \( NfI^{+/-} \) mice (FIGURE 5B). In addition, the voltage-dependence of \( I_h \) activation was shifted toward more hyperpolarized potentials in PV cells from \( NfI^{+/-} \) mice (FIGURE 5C). The half-activation potential for \( I_h \) in PV cells from \( NfI^{+/-} \) mice was -89.7±1.7 mV (n=7), compared to -84.7±1.1 mV (n=7, P=0.01) in wild-types. Under the same experimental conditions used, pyramidal cells showed no difference either in \( I_h \) amplitude (FIGURE 5E) voltage dependence activation (FIGURE 5F) in \( NfI^{+/-} \) mice as compared to wild-types. Furthermore, we evaluated \( I_h \)-dependent membrane properties in
CA1 hippocampal neurons in Nf1<sup>+/−</sup> mice and wild-type controls. The resting membrane potentials of PV cells recorded in Nf1<sup>+/−</sup> mice were on average more negative (Nf1<sup>+/−</sup>: -65.1±1.1 mV, n=14 and wt: -61.8±0.8 mV, n=9, P=0.042) and the input resistances higher (Nf1<sup>+/−</sup>: 289±15.0 MΩ, n=16 and wild-type: 258±9.5 MΩ, n=18, p=0.045) than those recorded from wild-types. In addition, consistent with down-regulation of I<sub>h</sub>, PV cells from Nf1<sup>+/−</sup> mice showed higher excitability, measured as number of action potential per depolarizing current pulse, compared with wild-type mice (FIGURE 5D). There was no significant change in the action potential voltage waveform or threshold indicating that the properties of voltage gated sodium and potassium channels are not substantially different in these mice. In consistent with lack of change in I<sub>h</sub> in pyramidal cells in Nf1<sup>8α<sup>y</sup>/3α<sup>y</sup></sup> mice, we found no significant difference in excitability of these cells between two groups (FIGURE 5G). These data further demonstrate that selective down-regulation of I<sub>h</sub> results in a reduction of I<sub>h</sub>-dependent membrane properties of PV cells in CA1 region.

**Example 6. Administration of Lamotrigine improve spatial memory in Nf1<sup>8α<sup>y</sup>/3α<sup>y</sup></sup> and Nf1<sup>+/−</sup> mice**

Altogether, our data show that similar to Nf1<sup>+/−</sup> mice, Nf1<sup>8α<sup>y</sup>/3α<sup>y</sup></sup> mice have spatial memory deficits which was associated with impairment of CA3-CA1 LTP and these arise from an increase in GABA-mediated inhibition as a consequence of higher membrane excitability of inhibitory neurons in CA1 hippocampal area. In addition, up-regulation of I<sub>h</sub> with Lamotrigine reduces the excitability of these neurons and rescues the LTP deficits in Nf1<sup>8α<sup>y</sup>/3α<sup>y</sup></sup> mice. Having shown the down-regulation of I<sub>h</sub> and higher excitability of CA1 interneurons in Nf1<sup>+/−</sup> mice, we ask whether impaired spatial learning and memory in Nf1<sup>8α<sup>y</sup>/3α<sup>y</sup></sup> and Nf1<sup>+/−</sup> mice can be rescued by Lamotrigine administration. All animals were handled for 3 days before the first injection. Mice were given intraperitoneal injection of vehicle or Lamotrigine (25 mg/kg) for 7 days before the first experiment and two-three hours before the first trial
of each day during behavioural experiments. All mice showed no deficiencies in swimming abilities, floating or thigmotaxic behavior and all groups improved their performance over training measured as escape latencies to a hidden platform. Spatial learning assessed by a probe trial given, and time searched in the target quadrant in which the platform was previously located (black column) was compared to the average of other quadrants. Vehicle treated NfI<sup>30a<sup>/30a* </sup></sup> mice showed no preference for the target quadrant, however, Lamotrigine improved the learning deficit of NfI<sup>30a* /30a* </sup> mice without an effect on the performance of wild-type littermates. There was a significant genotype by drug treatment interaction (two-way ANOVA, F<sub>1,43</sub> = 5.949, P = 0.019) for the time in the target quadrant (FIGURE 6A). Post hoc analysis indicates that Lamotrigine-treated NfI<sup>30a* /30a* </sup> mice (n=12) spent more time searching in the target quadrant than vehicle-treated NfI<sup>30a* /30a* </sup> mice (n=9, P<; FIGURE 6A). There was no significant difference between Lamotrigine-treated (n=14) and vehicle-treated (n=12) wild-type mice (P>0.05).

Similarly to the NfI<sup>30a* /30a* </sup> mice, we observed again a significant interaction of genotype and drug treatment (two-way ANOVA, F<sub>1,40</sub> = 5.188, P = 0.028), for the time in the target quadrant for the NfI<sup>+/−</sup> mice. Lamotrigine treatment significantly increased the time spent in the target quadrant in Lamotrigine-treated NfI<sup>+/−</sup> mice (n=10) as compared with vehicle-treated NfI<sup>+/−</sup> mice (n=9, FIGURE 6B). There was no significant difference between Lamotrigine-treated (n=14) and vehicle-treated (n=12) wild-type NfI<sup>+/−</sup> mice (P>0.05).

To investigate whether our finding also extended to non-hippocampal deficits we tested motor learning in NfI<sup>+/−</sup> mice. Nf1 patients have problems with fine motor skills, and we have previously shown that NfI<sup>+/−</sup> mice show rotarod deficits (van der Vaart et al., 2011, Genes Brain Behav 10, 404–409). Again we found a significant interaction between genotype and drug treatment (repeated ANOVA, F<sub>1,41</sub> = 7.721, P = 0.008); with no lamotrigine treated NfI<sup>+/−</sup> mice performing significant better than vehicle
treated $NfI^{++}$ mice. Again, no significant difference between vehicle and Lamotrigine-treated wild-type littermates was observed (FIGURE 6C).
Claims

1. A method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist.

2. Method according to claim 1, wherein said subject is a subject suffering from a developmental intellectual disability, preferably impaired spatial learning and memory due to inhibition of excitatory neurons in the brain, more preferably in a subject suffering from Neurofibromatosis type I (NF1) or Down syndrome.

3. Method according to claim 1 or 2, wherein said cognitive impairment is characterized by a low-average IQ, and problems with visuospatial skills, memory, language, executive functioning, and attention.

4. Method according to any one of claims 1-3, wherein said subject is between the age of 1 and 18 years, preferably 2-12 years.

5. Method according to any one of the preceding claims, wherein said HCN channel agonist is administered in a dosage of 0.1-100 mg/kg daily, preferably 5-15 mg/kg twice daily, preferably dosed between two-three hours before a cognitive training session.

6. Method according to any one of the preceding claims, wherein said administration is done by a route selected from oral, sublingual, pulmonary, nasal, intramuscular, intradermal, subcutaneous, intraperitoneal and intravenous, preferably oral.
7. Method according to any one of the preceding claims, wherein said HCN channel agonist is administered in a pharmaceutically acceptable carrier.

8. Method according to any one of the preceding claims, wherein said HCN channel agonist up-regulates the HCN channel-mediated non-selective cationic current (Ih) in inhibitory interneurons, preferably as determined in an Nf1+/− and/or an Nf1+/−/Ih mouse.

9. Method according to any one of the preceding claims, wherein said HCN channel agonist is selected from the group consisting of phenyltriazines, GABA analogs, (substituted) benzazepines, peptide neurotransmitters and (substituted) benzamides, preferably wherein said HCN channel agonist is selected from the group consisting of lamotrigine, analogues, derivatives and pharmaceutically acceptable salts thereof, more preferably lamotrigine.

10. HCN channel agonist for use in treating cognitive impairment in a subject.

11. HCN channel agonist for use according to claim 10, wherein said subject is a subject suffering from a developmental intellectual disability.

12. HCN channel agonist for use according to claim 10 or 11, wherein said subject suffering from Neurofibromatosis type I (NF1) or Down syndrome.

13. HCN channel agonist for use according to any one of claims 10-12, wherein said HCN channel agonist up-regulates the HCN channel-mediated non-selective cationic current (Ih) in inhibitory interneurons, preferably as determined in an Nf1+/− and/or an Nf1+/−/Ih mouse.
14. HCN channel agonist for use according to any one of claims 10-13, wherein said HCN channel agonist is selected from the group consisting of phenyltriazines, GABA analogs, (substituted) benzazepines, peptide neurotransmitters and (substituted) benzamides, preferably wherein said HCN channel agonist is selected from the group consisting of lamotrigine, analogues, derivatives and pharmaceutically acceptable salts thereof, more preferably lamotrigine.

15. HCN channel agonist for use according to any one of claims 10-14, wherein said HCN channel agonist is administered in a dosage of 0.1-100 mg/kg daily, preferably 5-15 mg/kg twice daily, preferably dosed between two-three hours before a cognitive training session.
FIG. 1

Input

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<th>HCN1-/- WT</th>
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IP with α-NF1

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<tr>
<th>NF1 A9A<em>9a</em></th>
<th>α-HCN1</th>
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α-NF1 | α-HCN1 |
FIG. 2A  FIG. 2E
FIG. 3A
FIG. 3B

Nf1^9a*/9a*

control

+ZD7288

Cumulative probability

Inter-event interval (ms)

0.0 0.2 0.4 0.6 0.8 1.0

0 200 400 600 800 1000

control

ZD7288

50 pA

500 ms
FIG. 3C

FIG. 3D
FIG. 4A

FIG. 4B
FIG. 6A
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/00 A61K31/53 A61P25/28

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)

A61K

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)

EPO-Internal, WPI Data, BIOSIS, EMBASE, SCISEARCH, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>WO 02/02630 A2 (SMITHKLINE BEECHAM PLC [GB]; STRIJBOS PAUL JOHANNES LEONARD [GB]; BATE) 10 January 2002 (2002-01-10) page 2, lines 22-27 page 2, line 35 - page 3, line 25 page 4, lines 6-9 page 6, lines 29-33 claims 1, 5</td>
<td>1,2,7,8, 10,11,13</td>
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<tr>
<td>X</td>
<td>WO 02/50300 A2 (MERCK &amp; CO INC [US]; FOLANDER KIMBERLY L [US]; LIU YUAN [US]; MCKENNA) 27 June 2002 (2002-06-27) page 27, line 14 - page 28, line 5 page 28, line 27 - page 29, line 2</td>
<td>1,2,5-8, 10,11, 13,15</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  *A* document defining the general state of the art which is not considered to be of particular relevance
  *E* earlier application or patent but published on or after the international filing date
  *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)
  *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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search: 22 February 2013

Date of mailing of the international search report: 18/04/2013

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Fax (+31-70) 340-3016

Authorized officer:
Cielen, Elsie
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<td>X</td>
<td>WO 2008/095221 A1 (GOSFORTH CT HOLDINGS PTY LTD [AU]; BIRD PHILIP [AU]) 14 August 2008 (2008-08-14) page 1, lines 4-9 page 4, lines 21-25 page 5, lines 29-32 page 9, lines 21-32 page 10, line 32 - page 12, line 2 page 14, lines 10-12 claims 1, 6, 10, 11, 20, 25</td>
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<td>KHAN ARIFULLA ET AL: &quot;Effect of lamotrigine on cognitive complaints in patients with bipolar I disorder.&quot;, THE JOURNAL OF CLINICAL PSYCHIATRY NOV 2004, vol. 65, no. 11, November 2004 (2004-11), pages 1483-1490, XP008160172, ISSN: 0160-6689 abstract page 1484, column 1, last paragraph - column 2, paragraph 2 page 1487, column 2, last paragraph - page 1488, column 1, paragraph 1 page 1489, column 1, paragraph 2 page 1489, column 2, paragraph 2</td>
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<td>AKHONDZADEH S ET AL: &quot;Does the addition of lamotrigine to risperidone improve psychotic symptoms and cognitive impairments in chronic schizophrenia?&quot;, THERAPY 200505 GB, vol. 2, no. 3, May 2005 (2005-05), pages 399-406, XP008160169, ISSN: 1475-0708 abstract page 401; table 1 page 403, column 2, last paragraph page 404, column 2, paragraph 2</td>
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Form PCT/ISA/210 (continuation of second sheet) (April 2005)
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INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of Item 2 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.:
   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:

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 No. III  Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

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

see additional sheet

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 fees, this Authority did not invite payment of additional fees.

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. [X] 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.:

   1-15(partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15(partially)

A method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist, which is a phenyltriazine, preferably selected from the group consisting of lamotrigine, analogues, derivatives and pharmaceutically acceptable salts thereof, more preferably lamotrigine.

HCN channel agonist for use in treating cognitive impairment in a subject, wherein the HCN agonist is a phenyltriazine, preferably selected from the group consisting of lamotrigine, analogues, derivatives and pharmaceutically acceptable salts thereof, more preferably lamotrigine.

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2. claims: 1-15(partially)

A method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist, which is a GABA analog.

HCN channel agonist for use in treating cognitive impairment in a subject, wherein the HCN agonist is a GABA analog.

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3. claims: 1-15(partially)

A method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist, which is a (substituted) benzazepine.

HCN channel agonist for use in treating cognitive impairment in a subject, wherein the HCN agonist is a (substituted) benzazepine.

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4. claims: 1-15(partially)

A method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist, which is a peptide neurotransmitter.

HCN channel agonist for use in treating cognitive impairment
in a subject, wherein the HCN agonist is a peptide neurotransmitter.

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5. claims: 1-15(partially)

A method of treating, preventing or ameliorating cognitive impairment in a subject, said method comprising administering to said subject a therapeutically effective amount of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel agonist, which is a (substituted) benzamide. HCN channel agonist for use in treating cognitive impairment in a subject, wherein the HCN agonist is a (substituted) benzamide.

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