There is provided methods to modulate biological or medical activities (functions), events and/or conditions. The biological or medical activities, events or conditions may include predisposition to, and onset and progression of neurological conditions, myelination and demyelination of neurons, and/or differentiation of neural stem cells. In particular, the present invention provides methods to modulate the expression and/or activity of amyloid precursor protein (APP) and/or its derivatives or fragments. The present invention also provides methods to modulate the expression and/or activity of molecules, such as sodium channels and/or TAG-1, which interact with APP and its derivatives or fragments.
**Modulation of Neural Activity and/or Condition**

**Field of the invention**

The present invention generally relates to the field of medical and biological science. In particular, the present invention relates to methods to modulate neural conditions, activities and/or events.

**Background of the invention**

Amyloid precursor protein (APP) is well known as a molecule that is involved in the pathogenesis of Alzheimer's disease (AD) and of other forms of dementia, such as Down's syndrome. The exact mechanisms by which APP contributes to the development of dementia are unknown but is it thought that cleavage of APP by secretases leading to formation of the smaller peptide, the beta amyloid (Aβ), is a crucial step.

Deposition of Aβ, has been suggested to be related to the pathology of AD and associated with other pathological markers of AD conditions such as cell death in the central nervous system (CNS), accumulation of amyloid plaques, and the appearance of neurofibrillary tangles. Reduction or prevention of Aβ deposition is widely believed to be desirable for slowing the progress of, or preventing the development of, AD. While reduction of Aβ deposition is thought to be desirable, there is no teaching or suggestion in the literature on how this may be achieved.

Previous research had suggested that release of Aβ may be activity dependent, but the reasons for this activity dependence were unknown. The release of component peptides of APP, including Aβ deposition, has also been thought to occur predominantly at synapses. However, although APP is enriched in axons and presynaptic terminals (Ferreira, 1993), its distribution and function along
myelinated neurons have yet to be elucidated. Further, although some of the potential interactions of APP had been explored, the interacting partners of APP were assumed to be synaptic proteins.

In AD patients, the entorhinal cortex, from where the perforant pathway originates, is one of the most severely affected areas, particularly at the earliest stages of the disease. Moreover, electrical activity within the pathway modulates interstitial fluid β-amyloid (Aβ) levels, which can be blocked by tetrodotoxin (TTX), a specific sodium channel blocker (Cirrito et al, 2005).

There is some evidence pointing to deficits in adult neurogenesis in AD and this has led to considerable interest in the role of APP in regulating stem cell function. However, the mechanisms by which APP modulate stem cell functions such as proliferation and differentiation remain unknown. In particular, the interacting or binding partners of APP in the stem cell niche are unknown.

As such, any contribution to the understanding of the role of APP in the brain will be useful and will add to knowledge of neural functions and/or events, as well as to treatment of neurological conditions.

Summary of the invention

The present invention addresses the problems above, and in particular, provides new methods to modulate at least one biological or medical condition, activity (function) and/or events.

Accordingly, the present invention provides a method of treating at least one medical condition in a subject, the method comprising modulating the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment. In particular, the modulating may be by decreasing
the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment.

The modulation of APP, its derivative or a fragment thereof may comprise modulating at least one molecule, protein, and/or peptide that interacts with amyloid precursor protein (APP), APP derivative or APP fragment. For example, the modulation may comprise modulating at least one cellular component that interacts with amyloid precursor protein (APP), APP derivative or APP fragment. The at least one cellular component may be selected from TAG-1, sodium channels, and sodium channel complex protein.

Accordingly, the modulation may comprise modulating at least one cellular component selected from Caspr2, NgR, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ NG2 and Go protein(s).

According to a further aspect, the modulating according to the invention comprises administering at least one pharmaceutically and/or therapeutically effective amount of amyloid precursor protein (APP), APP derivative or APP fragment. Preferably, the APP derivative or APP fragment is Aβ, soluble APP (sAPP), APP-695, AICD or VTPEER peptide. The modulating may comprise administering a pharmaceutically and/or therapeutically effective amount of at least one drug, protein, peptide, antibody or molecule that interacts with amyloid precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium channels, or sodium channel complex protein(s). The modulating may also comprise administering a pharmaceutically and/or therapeutically effective amount of at least one drug, protein, peptide, antibody or molecule that interacts with amyloid precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium channels, TAG-1 complex protein(s), sodium channels complex protein(s), Caspr2, NgR, potassium channels, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ and NG2. The modulating may also comprise administering a pharmaceutically and/or therapeutically effective amount of at
least one compound selected from the group consisting of TAG-1, anesthetic(s),
toxin(s) and Go protein(s). In particular, the modulating may comprise
administering a pharmaceutically and/or therapeutically effective amount of at
least one drug or molecule selected from local anaesthetic(s), veratridine,
batrachotoxin, scorpion toxins, sea anemone toxin, conus toxins, saxitoxin,
tetrodotoxin, antiepileptic drug(s), antiarrhythmic drug(s), antibodies to sodium
channel proteins, antibodies to Caspr, and peptide fragments of APP.

The modulation of amyloid precursor protein (APP), APP derivative or APP
fragment may be to prevent and/or reduce beta amyloid (Aβ) deposition,
improve and/or facilitate nervous tissue repair, improve and/or facilitate
myelination, to modulate stem cell function(s) and/or to modulate sodium
channel function(s). The at least one medical condition may be selected from
the group consisting of Alzheimer's disease, multiple sclerosis, brain injury,
spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative
disorders and demyelination disorders. The modulating may result in
modulation of axonal conduction of action potential(s) for the treatment of pain
and/or epilepsy. The modulating may result in the induction of analgesia and/or
local anaesthesia.

The APP derivative may be soluble APP (sAPP) and the APP fragment may be
beta amyloid (Aβ).

The present invention also provides a method of detecting and/or quantitating
the presence of, predisposition to, and/or severity of, a neurological condition in
a subject, the method comprising: (a) providing at least one sample from a
subject; (b) determining the expression or activity of amyloid precursor protein
(APP), APP derivative or APP fragment; (c) comparing the expression or activity
of amyloid precursor protein (APP), APP derivative or APP fragment, with that
of at least one control, a difference in expression and/or activity indicating the
presence of, or predisposition to, and/or severity of a neurological condition in the subject.

There is also provided a method of monitoring the efficacy of a treatment for a neurological condition in a subject, the method comprising: (a) providing at least two samples from a subject, each sample obtained at different time points; (b) determining the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment; and (c) comparing the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment, in the at least two samples.

There is also provided a method of prognosticating the outcome of a neurological condition in a subject, the method comprising: (a) providing at least one sample from a subject; (b) determining the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment; (c) comparing the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment, with that of at least one control, a difference in expression and/or activity (function) indicating the prognosis of a neurological condition in the subject.

There is also provided a method of inducing analgesia and/or anesthesia in a subject, the method comprising modulating the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment. The modulating may comprise decreasing the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment.

The present invention also provides an in vitro or in vivo method of modulating proliferation, differentiation and/or function (activity) of at least one stem cell, the method comprising modulating the expression and/or activity (function) of amyloid precursor protein (APP), APP derivative or APP fragment, in the at least one stem cell. The present invention also provides a method of detecting and/or quantitating the presence of, predisposition to, and/or severity of, at least
one condition related to beta amyloid (Aβ) deposition and/or sodium channel
dysfunction in a subject, the method comprising: (a) measuring the nerve
and/or axon conduction velocity in the subject; (b) comparing the nerve and/or
axon conduction velocity with that of at least one control, a difference in velocity
indicating the presence of, or predisposition to, and/or severity of at least one
condition related to Aβ deposition and/or sodium channel dysfunction in the
subject. The method may be for detecting and/or quantitating the presence of,
predisposition to, and/or severity of Alzheimer’s disease (AD).

The present invention also provides an in vitro or in vivo method of modulating
at least one activity (function) of amyloid precursor protein (APP), APP
derivative or APP fragment, comprising administering at least one drug, protein,
peptide, antibody or molecule that interacts with amyloid precursor protein
(APP), APP derivative or APP fragment, TAG-1, sodium channels, or sodium
channel complex protein(s). The modulating may comprise administering a
pharmaceutically and/or therapeutically effective amount of at least one
compound selected from the group consisting of TAG-1, anesthetic(s), toxin(s)
and Go protein(s). The method may be for treating at least one condition
selected from the group consisting of Alzheimer’s disease, multiple sclerosis,
brain injury, spinal cord injury, axonal injury-related disorders, epilepsy,
neurodegenerative disorders and demyelination disorders.

The present invention also provides an in vitro or in vivo method of modulating
sodium channel(s) activity(ies) (function/s) comprising administering at least
one drug, protein, peptide, antibody or molecule that interacts with amyloid
precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium
channel(s), or sodium channel complex protein(s). The method may be for
modulating at least one APP activity (function), and/or for the treatment of at
least one neurological disorder. The method may be for the treatment of acute
and/or chronic pain disorder(s), induction of analgesia, treatment of epilepsy
and/or convulsion(s), treatment of disorder(s) of the rhythm of the heart, and/or induction of anaesthesia and/or analgesia.

The present invention also provides at least one pharmaceutical composition for treating at least one APP and/or sodium channel related medical condition comprising at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s), toxin(s) and Go protein(s); and optionally at least one pharmaceutically acceptable diluent, carrier and/or excipient. The medical condition may be selected from the group consisting of Alzheimer’s disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders and demyelination disorders.

The present invention also provides use of at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s), toxin(s) and Go protein(s), for the preparation of a medicament for the treatment of an APP and/or sodium channel related medical condition.

The present invention also provides a compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s), toxin(s) and Go protein(s) for the treatment of an APP and/or sodium channel related medical condition.
The present invention also provides a kit comprising at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s) and toxin(s), for the detection and/or diagnosis of at least one APP and/or sodium channel related medical condition and/or for the treatment of at least one APP and/or sodium channel related medical condition.

Preferably, the APP derivative or fragment is Aβ, soluble APP (sAPP), App-695, AICD or VTPEER peptide.

Brief description of the figures

Figure 1 shows that APP is expressed by axons during myelination. It also shows the interaction between APP and TAG-1, Caspr2, NgR, and K+ channels.

Figure 2 shows that APP is expressed at nodes of Ranvier. Interaction with Sodium channels places APP in the context of TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, Ompg, RPTOβ and NG2 complexes.

Figure 3 shows that TAG-1 is expressed in the stem cell niche and that APP interacts with TAG-1.

Figures 4-8 show that APP locates at NORs in the CNS.

Figure 4 shows that APP was detected at NORs in the spinal cord, a-k: Normal adult rat spinal cord were prepared for double IF, using polyclonal antibodies to APP (green; a-k) and monoclonal antibodies against Kv1.2 (Kch; red; a d, f, and g) or Tn-R (red; b, h j, and k) or Na+ channels (Nach; red; c), respectively, g and k: Orthogonal slices. Scale bars: 10 µm in a-c, f, g, j, and k.
Figures 5(a, b) show that APP evenly distributed along sciatic nerve. Normal adult rat sciatic nerves (SN) were prepared for double IF, using polyclonal antibodies to APP (Green) and monoclonal antibodies against NF200 (red; a) or Kv1.2 (red; b), respectively. Scale bars: 10 µm in a and b.

Figures 5(c, d, e) show disappearance of APP clustering in the spinal cord of APP knock-out mice. Adult spinal cord from APP knock-out mice (KO) was prepared for double IF, using polyclonal antibodies to APP (Green; a) or Caspr (Green; b and c) and monoclonal antibodies against Tn-R (red; a and b) and Nach (red; c), respectively. Scale bars: 10 µm in a, b.

Figure 6 shows the results of immunohistochemical studies of longitudinal sections of rat spinal cords at P6, P10, P15, and P21. Double labellings using antibodies against pan-Na+ channels (Nach, red; a) and K+ channel (Kch, red; b to d) showed that APP clustering commences at P10 and becomes prevalent by P15. Insert boxes show images with higher magnification from the respective lower magnification images.

Figure 7 shows quantification of double IFs of APP and K+ channels performed in spinal cords. Nodal APP labellings in relation to Kv1.2 in spinal cord were counted (n = 200) from at least three animals (e). The ratios were detected as 52.08 ± 7.29% at P10, 66.94 ± 0.9% at P15, 68.97 ± 2.3% at P21, and 90.16 ± 3.05% at 2 months. Scale bars: 10 µm in a-d.

Figure 8 shows quantification of double IF labelling of APP and K+ channels performed in spinal cords. Nodal APP labellings in relation to Kv1.2 in spinal cord were counted (n = 200) from at least three animals. The ratio was detected as 90.16 ± 3.05%.

Figures 9-12 show myelination in APP knock-out and transgenic mice (Scale bars: 0.5 µm in Aa, Ab, Ca, and Cb; 5 µm in Ba, Bb. Error bars represent +SEM in Ac, Bc, and Cc. * * p < 0.001).
Figure 9 shows the results of EM studies in cross sections revealed the ultrastructure of myelin sheaths in spinal cords (SC) of wild-type littermates (WT; a) versus APP knock-out mice (KO; b) (all aged 3 months). The myelin thickness as indicated by g ratio (c) was significantly reduced in APP knock-out mice, in comparison with wild-type littermates.

Figure 10 shows the results of EM studies on the ultrastructure of myelin sheaths in sciatic nerve (SN). Cross sections revealed that wild-type littermates (WT; a) versus APP knock-out mice (KO; b) (all aged 3 months). The myelin thickness as indicated by g ratio (c) was significantly reduced in APP knock-out mice, in comparison with wild-type littermates.

Figure 11 shows the results of EM studies in cross sections revealed the ultrastructure of myelin sheaths in spinal cords (SC) of wild-type littermates (WT; a) versus APP transgenic mice (TG; b) (all aged 3 months). The myelin thickness as indicated by g ratio (c) was significantly increased in APP transgenic mice, in comparison with wild-type littermates.

Figure 12 shows the results of APP knock-out and overexpression showed the opposite effects on both axon (a) and fiber (b) diameters and the myelin thickness as indicated by g ratio (c), in mice after normalizing with wild-type littermates, respectively.

Figures 13-16 show that APP modulates the expression of Na+ channel Nav1.6 in *Xenopus* oocytes. Two-electrode voltage clamp recordings of Na+ currents in *Xenopus* oocytes expressing Nav1.6 in oocytes expressing: Figure 13, the α subunit alone and with APP; Figure 14, the α + β1 subunits without and with APP; Figure 15, the α + β2 subunits without and with APP; and Figure 16, α + β1 + β2 subunits without and with APP Traces (a) to (d).
Figure 13 illustrates the effects of APP on voltage-gated sodium channels.

a. Normalized current-voltage relationships of currents in oocytes expressing Nav1.6 α subunits alone (open squares) and together with APP (closed circles).

b. Activation of sodium currents in oocytes expressing Nav1.6 α subunits alone (open squares) and together with APP (closed circles). The activation curves were fitted by a Boltzmann equation, \( \frac{G_{\text{Na}}}{G_{\text{Na, max}}} = \frac{1}{1 + \exp\left(V_{1/2} - V_m\right)/k} \), where \( V_{1/2} \) is the voltage of half-maximal Na\(^+\) conductance and \( k \) is a slope factor.

c. Normalized inactivation of sodium currents in oocytes expressing Nav1.6 α subunit alone (open squares) and together with APP (closed circles). The inactivation data were fitted to a Boltzmann function, \( \frac{I_{\text{Na}}}{I_{\text{Na, max}}} = \frac{1}{1 + \exp\left(V_m - V_{1/2}\right)/k} \), where \( V_{1/2} \) is the voltage of half-maximal availability and \( k \) is a slope factor.

d. Recovery from inactivation was measured using a two-pulse protocol with a variable interval in oocytes expressing the Nav1.6 α subunit alone (open squares) and together with APP (closed circles). The data were fitted with a single rising exponential function.

e. Normalized current-voltage relationships of sodium currents in oocytes expressing Nav1.5 α subunit alone (open squares) and together with APP (closed circles).

f. Normalized current-voltage relationships of sodium currents in oocytes expressing Nav1.6 α subunit alone (open squares) and together with control injection of cRNA for the reverse APP sequence (closed circles).

Insert: Representative examples of sodium current responses elicited by depolarizing voltage steps.

For figures 14-16

a shows representative examples of Na\(^+\) current responses elicited by depolarizing voltage steps.
b shows normalized current-voltage relationships of Na+ currents in oocytes expressing Na+ channels alone (open circles) and together with APP (closed circles).

c shows activation of Na+ currents in oocytes expressing Na+ channels alone (open circles) and together with APP (closed circles). The activation curves were fitted by the Boltzmann equations $G_{Na}/G_{Na,max} = 1/[1+exp(V1/2 - V_m)/k]$ and $I_{Na}/I_{Na,max} = 1/[1+exp((V_m-V1/2)/k)$, respectively, where $V1/2$ is the voltage of half-maximal Na+ conductance and half-maximal availability, respectively, and $k$ is a slope factor.

d shows normalized inactivation of Na+ currents in oocytes expressing Na+ channels alone (open circles) and together with APP (closed circles). The inactivation data were fitted to a Boltzmann function $G_{Na}/G_{Na,max} = 1/[1+exp(V_m - V1/2)/k]$ and $I_{Na}/I_{Na,max} = 1/[1+exp((V_m-V1/2)/k)$, as described above.

e shows recovery from inactivation measured using a two-pulse protocol with a variable interval in oocytes expression sodium channels alone (open circles and together with APP (solid circles).

Figures 17-18 show electrophysiological properties in APP-knock-out mice.

Figure 17 shows whole cell recordings of voltage-gated Na+ currents in acutely dissociated DRG neurons, a, Na+ current responses elicited by depolarizing voltage steps in a neuron from an APP knockout mouse (top). Normalized current-voltage curves of peak Na+ currents in wild-type mice (solid circles) and APP knockout mice (open circles). pF = picofarads, b, activation (circle symbols) and steady-state inactivation (triangle symbols) curves of Na+ currents in neurons from wild-type mice (dash line) and APP knockout mice (solid line). The activation and inactivation curves were fitted by the Boltzmann equations $G_{Na}/G_{Na,max} = 1/[1+exp(V1/2 - V_m)/k]$ and
I\textsubscript{Na}/I\textsubscript{Na, max}=1/[1+exp((V\textsubscript{m}-V\textsubscript{1/2})/k), respectively, where V\textsubscript{1/2} is the voltage of half-maximal Na\textsuperscript{+} conductance and half-maximal availability, respectively, and k is a slope factor, c; recovery from inactivation measured using a two-pulse protocol with a variable interval in neurons from wild-type mice (open circles) and APP knockout mice (solid circles). Data points indicate the amplitude of the second Na\textsuperscript{+} current response after normalization to the amplitude of the first response. Data in recordings from 9 neurons from four wild-type mice and 19 neurons from five APP knockout mice in b-c summarize recordings from 16 neurons from eight wild-type mice and 24 neurons from eight APP knockout mice.*, p < 0.05. **, p < 0.01.

Figure 18 shows the determination of conduction velocities of compound action potentials in APP-knock-out and wild-type mice. Representative CAPs from spinal cord recordings of APP knock-out (KO) and wild-type (WT) mice. Records were taken at 24°C, 32°C and 37°C for spinal cord (a). The conduction velocity measured in wild-type mouse spinal cord (a; n =8) was consistently higher than that seen in APP knock-out mice (a; n =12) at all temperatures measured. The conduction velocity measured in wild-type mouse sciatic nerves (b; n = 6) was slightly higher than that seen in APP knock-out mice (a; n =11) at all temperatures measured, but the difference was not significant. Significance values were calculated using the Student's t test and were as follows: 24°C, p < 0.05; 32°C, p < 0.05; and 37°C, p < 0.005. Error bars represent ±SEM.

Figure 19A shows APP expression during development. Protein extracts of rat whole brains at various postnatal developmental stages (PO, P5, P7, P8, P10, P15, P21, P30 and adult) were subjected to Western blotting analyses using antibodies against APP and γ-tubulin (as loading control). Figure 19B shows that APP clustering was disrupted in the spinal cord of PLP transgenic mice (arrow). APP is still clustering at node of Ranvier in the unaffected myelinated fiber (arrowhead). Adult spinal cords from both mice were prepared for double
IF, using polyclonal antibodies to APP (green) and monoclonal antibodies against K+ channels (red) respectively. Scale bars: 10 µm.

Figures 20-22 show effects on axon and fiber diameters. (Note that increment of g ratio in KO mice is significant in almost all axons. ** p < 0.001; * p < 0.05. Error bars represent ±SEM.)

Figure 20 shows that both axon (a) and fiber (b) diameters were significantly reduced in APP knock-out mice, in comparison with wild-type littermates. The distribution of the g ratio, representing the myelin thickness, in the spinal cord (c) was plotted against different axon diameters, comparing APP KO to WT mice.

Figure 21 shows the distribution of G ratio representing the myelin thickness of sciatic nerve was plotted against different axon diameters, comparing in APP KO and WT mice.

Figure 22 shows that both axon (a) and fiber (b) diameters were significantly increased in APP transgenic mice, in comparison with wild-type littermates.

Figure 23 shows effect of co-expression of APP on peak Na+ currents of oocytes expressing the Nav1.6 with a, α subunit; b, α + β1 subunit; c, α + β2 subunit; and d, α + β1 + β2 subunit; ** p < 0.005.

Figure 24 shows a western blot analyses of protein extracts from the brains of adult APP+/+, APP+/−, and APR−/− mice. Antibodies used were directed against Nav1.6 and γ-tubulin (as loading control).

Figure 25 shows a schematic representation of APP (Full-length APP695; SEQ ID NO: 2, cDNA; SEQ ID NO: 3, peptide), APP intracellular domain (AICD50; SEQ ID NO: 4, cDNA; SEQ ID NO: 7, peptide), APP C31 domain (C31; SEQ ID NO: 8, cDNA; SEQ ID NO: 10, peptide), APP Go protein binding domain (GOBD; SEQ ID NO: 11, cDNA; SEQ ID NO: 14, peptide), Fe65 binding domain
(Fe65BD; SEQ ID NO: 15, cDNA; SEQ ID NO: 18, peptide) and VTPEER peptides (Val^{667}-Arg^{672}; SEQ ID NO: 19, cDNA; SEQ ID NO: 22, peptide).

Figure 26 shows the effects of distinct APP intracellular domains and peptides on voltage-gated sodium channels.

Normalized current-voltage relationships of currents in oocytes expressing Nav1.6 α subunits alone (open squares) and together with APP intracellular domain (AICD50; a; closed circles), APP Go protein binding domain (GOBD; b; closed circles), Fe65 binding domain (Fe65BD; c; closed circles), APP C31 domain (C31; d; closed circles), and VTPEER peptide (VTPEER; e; closed circles). Normalized current-voltage relationships of currents in the cells expressing Nav1.6 α subunits before (f; open squares) and after (f; closed circles) VTPEER treatment. Insert: Representative examples of sodium current responses elicited by depolarizing voltage steps.

Figure 27 shows the effects of Go protein on the APP-induced sodium current expression.

a. Sodium channels and APP alone (open squares) and together with a G203T dominant negative mutant of the Go protein α subunit (GoDN; closed circles).
b. Sodium channels and AICD alone (open squares) and together with GoDN (closed circles).
c. Sodium channels and the Go protein-binding domain (GB) of APP alone (open squares) and together with GoDN (closed circles).
d. Sodium channels and the Fe65 binding domain (Fe65BD) of APP alone (open squares) and together with GoDN (closed circles).
e. Sodium channels and the C31 domain (C31) of APP alone (open squares) and together with GoDN (closed circles).
f. Sodium channels and the VTPEER peptide of APP alone (open squares) and together with GoDN (closed circles).
Figure 28 shows the effects of APP distinct domains and peptides on Nav1.6 electrophysiological properties.

Activation of sodium currents in oocytes expressing Nav1.6 α subunits alone (open squares) and together with AICD (a), Go binding domain (GB; d), C31 (g), and VTPEER peptides (j; closed circles), respectively. The activation curves were fitted by a Boltzmann equation, $G_{N_a} / G_{N_a,max} = 1/[1 + \exp(V_i / V_{1/2} - V_m)/k]$, where $V_{1/2}$ is the voltage of half-maximal Na$^+$ conductance and k is a slope factor.

Normalized inactivation of sodium currents in oocytes expressing Nav1.6 α subunit alone (open squares) and together with AICD (b), Go binding domain (GB; e), C31 (h), and VTPEER peptides (k; closed circles), respectively. The inactivation data were fitted to a Boltzmann function, $\ln(Na/Na,max) = 1/[1 + \exp(Vm - V_{1/2})/k]$, where $V_{1/2}$ is the voltage of half-maximal availability and k is a slope factor.

Recovery from inactivation was measured using a two-pulse protocol with a variable interval in oocytes expressing the Nav1.6 α subunit alone (open squares) and together with AICD (c), Go binding domain (GB; f), C31 (i), and VTPEER peptides (l; closed circles), respectively. The data were fitted with a single rising exponential function.

Figure 29 shows the relationship between the amplitude of normalized sodium currents and the APP concentration.

Nav1.6 was co-expressed with full length APP in various molar ratios in oocytes. The graph shows the normalized mean peak sodium current amplitude ± SEM in oocytes injected with the indicated concentrations by molar ratio of Nav1.6 and APP cRNA. The effect of APP on the sodium current remained
constant over a wide range of APP concentrations suggesting that the effect on the sodium current does not depend upon a binding of the sodium channel with APP as a beta subunit or accessory protein. The data were obtained from between 18 and 42 oocytes from 4 frogs at each concentration.

Figure 30 illustrates that APP and TAG-1 are binding partners.

A. Cell adhesion assay. TAG-1 transfected CHO cells (a), but not F3 transfected CHO cells (d) and CHO cells (e), adhered to APP-Fc protein spots. Adhesion of TAG-1 transfected CHO cells to APP-Fc was blocked by antibodies against TAG-1 (b) and APP (c). APP transfected CHO cells (f, g, h, j and l), but not CHO cells (i, k, m, and n), adhered to spots coated with TAG-1-Fc protein (f), TAXIg-GST (g), and TAXFNII-GST (l), but not GST (n). Adhesion of APP transfected CHO cells to TAG-1-Fc was blocked by antibodies against TAG-1 (g) and APP (h). Bar in n is 20 µm (for a-n). ** p<0.001.

B. Quantification of TAG-1 transfected CHO cells adhering to APP-Fc substrate, APP transfected CHO cells adhering to TAG-1-Fc substrate, and the effects of blocking with antibodies against TAG-1 and APP. Error bars represent standard deviations.

Figure 31 shows that (a) APP and TAG-1 associate as a protein complex. Co-immunoprecipitation of APP with TAG-1. Mouse brain lysates were co-immunoprecipitated using anti-TAG-1 antibodies and non-immune IgG and probed with anti-APP antibody. Reciprocal assays used anti-APP antibody or APP-Fc to capture the protein complex and anti-TAG-1 antibodies to detect the binding partner. (b) GST Pulldown assay to analyze the interaction between TAX and APP. Mouse brain, APP/CH0, and CHO cell lysates were precipitated using TAXFNIII-GST, TAXIg-GST, and GST and probed with anti-APP antibody.
Figure 32 shows that AICD transcriptional activity in TAG-1- and TAX-transfected CHO cells and expression of APP and TAG-1 in the fetal neural stem cell niche. A. (a) CHO cells and L1 (CHOL1), TAX (CHOTAX) and TAG-1 (CHOTAG1) transfected CHO cells were transiently co-transfected in 24-well culture dishes with pG5E1 B plasmid, APP-Gal4-responsive luciferase reporter gene, Fe65 plasmid, and luciferase internal control plasmid. Normalized luciferase activities in whole-cell lysates were determined in triplicate and expressed relative to activity in lysates prepared from CHO cells. (b) AICD transcriptional activity in TAG-1 transfected CHO cells was significantly reduced by a γ-secretase inhibitor (2 µM and 4 µM). ** p<0.001. Error bars represent standard deviations.

Figure 33 shows double immunostaining for nestin (red, a, a', d, and d'; c, c', f, and f are the merged images) and APP (green, b, b', c, and c') or TAG-1 (green, e, e', f, and f') in the walls of the lateral ventricles in E14 mouse brain. Double immunostaining for APP (green, h and h'; i and i' are the merged images) and TAG-1 (red, g, g', i, and i') in the walls of the lateral ventricles in E14 mouse brain. Bars in c, f, and i are 50µm (for a, b, d, e, g, and h); Bars in c', f', i' are 20 µm (for a', b', d', e', g' and h').

Figure 34 shows the expression of APP and TAG-1 in neural progenitor cells (NPCs). NPCs isolated from the lateral ventricles of E14 mouse brain were double-stained for APP (green; a' and red; c' and e') and/or TAG-1 (green; b' and e' and red; d') and with neural precursor cell markers, nestin (red; a and b) or Sox2 (green; c and d). a" to e" are merged images. Mouse brain samples and the NPCs lysate were blotted with antibodies against TAG-1 and APP, respectively (f). Bars are 20 µm in a" (for a and a''); b" (for b and b''); c" (for c and c''); d" (for d and d''); and e" (for e and e').

Figure 35 shows the neurogenesis in APP and TAG-1 deficient mice.
A. NPCs were isolated from APP +/+ mice (APP WT) and APP -/- mice (APP KO). After 7-8 days in vitro culture differentiation, the cells were double-stained for TUJ 1 (a and b) or MAP2 (d) and DAPI (a and b). The number of TUJ 1 (c) and MAP2 (d) positive cells were counted and expressed as a percentage of the total number of DAPI positive cells. Bar in b is 100 µm (for a).

B. NPCs isolated from TAG-1 +/+ mice (TAG-1 WT) and TAG-1 -/- mice (TAG-1 KO) were double-stained for TUJI (a and b) or MAP2 (d) and DAPI (a and b). The number of TUJ 1 (c) and MAP2 (d) positive cells were counted and expressed as a percentage of the total number of DAPI positive cells. Bar in b is 100 µm (for a).

** p<0.001. Error bars represent standard deviations.

Figure 36 shows TAG-1 triggered AICD transcriptional activity in NPCs, neurogenesis in TAG-1/APP double deficient mice and a putative model of the TAG-1/APP pathway.

A. NPCs isolated from APP-/-/TAG-1-/-mice were transiently co-transfected with pG5E1B plasmid, a APP-Gal4-responsive luciferase reporter gene, Fe65 plasmid, and luciferase internal control plasmid (a). The transfected NPCs were cultured in 24-well culture dishes substrate-coated with laminin, F3-Fc, LI-Fc, and TAGI-Fc as indicated. Normalized luciferase activities in whole-cell lysates were determined in triplicate and expressed relative to the activity in lysates prepared from laminin treated cells. AICD transcriptional activity in APP-/-/TAG-1-/- NPCs was significantly reduced by a γ-secretase inhibitor (2 µM and 4 µM, respectively; b). ** p<0.001. Error bars represent standard deviations.

B. NPCs isolated from APPWTAG- 1+/+ mice (WT) (a) and TAG-1 -/-/APP-/- mice (b) were double-stained for TUJI and DAPI. The percent of TUJI/DAPI positive cells were counted (e). ** p<0.001. Error bars represent standard deviations. Bar in b is 100 µm (for a).
Figure 37 shows a putative model of the TAG-1/APP pathway. TAG-1 interacts with APP on the cell surface to activate the APP signaling pathway through γ-secretase-dependent cleavage. This is followed by the processing of APP and release of AICD into cytoplasm, where AICD may bind with Fe65 or other modifiers. The complex further translocates to the nucleus, where it interacts with other transcription-related proteins, such as Tip60, and thereby activates target genes. Thus, neurogenesis is negatively modulated by AICD transcriptional activity triggered by the TAG-1/APP interaction.

Detailed description of the invention

Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content of such bibliographic references is herein incorporated by reference.

Definitions

Medical or biological activity (function), event and/or condition - These are events, activities (functions) and/or conditions in humans and/or animals. Since such events, activities (functions) and/or conditions are homologous or equivalent in both humans and animals, and/or share many similar causations, processes or pathways, the present invention may be applicable to either humans or animals or to both. Accordingly, events, activities (functions) and/or conditions referred to in humans also refer to homologous or equivalent events, functions or conditions in animals.

Neural/neurological activity (function) - Neural activities (functions) are activities (functions) controlled by the nervous system. These include the functioning of the nervous system, sensing of changes in both the internal and external environments, the interpretation of those changes and the coordination of responses. Neural activities (functions) may comprise of one or more neural events. For the purpose of the present invention the terms "activity" or
"function" are assumed to have the same meaning and are used interchangeably. Further, any reference to the term in singular form, for example, "activity" or "function" also encompasses the plural term, that is, "activities" or "functions".

Neural event - events taking place in the nervous system such as myelination, differentiation, growth, changes and cell death and includes generation and propagation of action potentials. Neural events can either be normal or abnormal.

Neurological condition - the state of a part of, or the entire nervous system. It usually refers more to abnormal or disease conditions than normal conditions.

APP, APP derivative or APP fragment - For ease of description, in the present application, any reference to APP may also apply to any APP derivative as well as to any APP fragment which maintain the same, similar, equivalent or comparable biological activity (function) of APP.

APP expression and/or activity - APP expression related to the expression of the APP encoded by the APP gene. APP activity (also indicated as "function" as defined above) relates to the APP biological activity (function). In particular, to the neural/neurological activity (function), as defined above, of APP. For instance, to the APP capacity to interact with other cellular proteins and/or exogen drug(s) or compound(s) and to modulate their or its activity (function). For example, it relates to APP capacity to modulate the activity (function) of sodium channel(s) and TAG-1. As a further example, the APP activity (function) also encompasses the cleavage of APP and the formation of the beta amyloid (Aβ).

Modulating - varying or changing a state, level, system or event. For example, in certain aspects, modulation may be by increasing or decreasing the level or expression of a molecule in a system.
Expression - The conversion of a gene into a gene product which may be RNA or protein. Accordingly, the expression of a gene product may be controlled at two levels: at the transcription level or at the translation level, to obtain a change in the prevalence of the molecular product.

Cellular component - Any part of a cell including structural and non-structural components.

Protein - A polymer of amino acids linked via peptide bonds and which may be composed of one or more polypeptide chains. A protein may also have other non-amino acid components such as carbohydrates or lipids associated with it or may be post-translationally modified (e.g. by glycosylation or phosphorylation). A protein may be processed in or outside the cell (ie in vivo or in vitro) to obtain derivatives or cleaved to form one or more protein fragments. A protein may be purified from natural sources or artificially synthesized. A protein under the present invention includes proteins that are substantially identical to it. By "substantially identical", it is meant a protein exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% sequence identity to the reference protein.

Homologous - For the purpose of the present invention, a molecule is said to be homologous to a reference molecule if that molecule is able to exert or elicit a similar response in a cell or biological system as that exerted or elicited by the reference molecule. For example, a synthetic molecule having an active site similar to that of a biologically-active reference molecule may be able to exert or elicit a similar response in a cell or biological system without the synthetic molecule having to possess a similar overall chemical structure as that of the reference molecule.

Interacts/interaction - Two biological molecules are said to interact with one another when either of them causes a change in the other molecule or to itself. The change may be a physical change or a change in biological properties.
Accordingly, the interaction may be direct wherein the two molecules come into direct physical contact or the interaction may be indirect when the interaction is via one or more intermediate molecules.

Sodium channel complex refers to a complex comprising molecules which interact with sodium channel. The molecules include but are not limited to Caspr2, NgR, potassium channels, Tn-R, Tn-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ, NG2 and Go protein(s).

TAG-1 complex refers to a complex comprising molecules which interact with TAG-1. The molecules include but are not limited to APP, Caspr2, NgR, K+ channels, Tn-R, Tn-C, MAG, NogoA, phosphacan, F3, OMgp, RPTPβ, NG2 and Fe65.

Description

Myelinated axons in the central nervous system (CNS) are characterized by their segmental structures: the node of Ranvier (NOR), paranode, juxtaparanode, and intemode. The present inventors have surprisingly found that, contrary to previously held beliefs, APP is localized and/or aggregated at the nodes of Ranvier (NORs) of axons in the central nervous system (CNS), but not the peripheral nervous system (PNS), and interacts with sodium channels and/or TAG-1. This localization and interaction with TAG-1 reveals novel activities (functions) for APP. In particular, it reveals novel activities (functions) for APP in myelination. The interaction with at least sodium channels and/or TAG-1 also provides novel target(s) for the modulation of APP function. It also provides novel targets for the modulation of Aβ deposition in Alzheimer’s disease and other diseases associated to the modulation of APP, sodium channels or TAG-1. In particular, it provides novel target(s) for diseases related with Aβ deposition. Thus, according to the method of the present invention, the inventors provide method(s) to modulate biological and/or medical events, activities (functions) and/or conditions, such as neural activities (functions),
events and/or neurological conditions, by modulating the activity (function) and/or expression of APP, its derivative or a fragment thereof, or a molecule that interacts with APP, its derivative or a fragment thereof.

The inventors also found that TAG-1 is an interacting partner of APP in the stem cell niche and accordingly provides novel target(s) for modulation of stem cell activity (function). In addition to directly revealing sodium channel(s) and TAG-1 as target(s) for modulating APP activity (function) in axons and/or stem cells, association of APP with TAG-1 and sodium channel(s) reveals numerous other targets for modulation of APP by the known association of TAG-1 and sodium channels with protein complexes. These protein complexes involve numerous molecules including Caspr2, NgR, K+ channels, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ and NG2. Additionally, the findings also suggest novel means of modulating sodium channel activity (function) and hence axonal conduction that may have application in treatment of pain, in local anaesthesia, or in the treatment of neurological disorders such as epilepsy.

In particular, the inventors have found that:

(a) APP is localized at the nodes of Ranvier in the myelinated axons of the central nervous system.

(b) APP interacts with sodium channels.

(c) APP interacts with TAG-1.

(d) TAG-1 is localized on stem cells and in the stem cell niche in the brain.

The finding revealed novel targets for modulation of APP and its functions in health and disease (e.g. to treat AD or to manipulate stem cells) and novel activities (functions) of APP in myelination (e.g. in the treatment of brain or
spinal injury and MS). The invention also provides novel target(s) for modulating sodium channel activity(ies) (function/s) (e.g. to achieve analgesia, local anaesthesia, or in the treatment of neurological disorders such as epilepsy).

As mentioned above, the release of component peptides of APP, including Aβ deposition, had been thought to occur at synapses. Although some of the potential interactions of APP had been explored, the interacting partners of APP were believed to be synaptic proteins. Therefore there was no knowledge that APP interacted with sodium channels and no knowledge of the intra- and intercellular protein-protein interactions of APP with TAG-1. In addition to forming Aβ after cleavage by secretases, APP can be cleaved by to release soluble APP (sAPP). The exact activities (functions) of sAPP were unknown but it has been suggested that sAPP may act as trophic factor. Together with evidence that there are deficits in adult neurogenesis in AD, this has lead to considerable interest in the role of APP in regulating stem cell function. However, the mechanisms by which APP modulate stem cell activities (functions) such as proliferation and differentiation was unknown. In particular, the interacting or binding partners of APP in the stem cell niche were unknown.

As shown in Figure 1, the present inventors have found that APP is expressed by axons during myelination and interactions with TAG-1 places APP in the TAG-1, Caspr2, NgR, K+ channel complex and reveals targets for modulation of myelination (e.g. in treatment of multiple sclerosis or spinal injury). Figure 2 shows that APP is expressed at nodes of Ranvier and interactions with sodium channels places APP in the context of TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ and NG2 complexes and reveals both novel targets for modulation of APP activity (function) including amyloid deposition (e.g. in treatment of Alzheimer's disease) and novel targets for sodium
channel modulation (e.g. in analgesia, local anaesthesia and treatment of epilepsy). Figure 3 shows that TAG-1 is expressed in the stem cell niche and that APP interacts with TAG-1 reveals novel targets for modulation of stem cell activity (function) (e.g. in cyotherapeutic brain repair).

Accordingly, the present inventors provide novel means to modulate at least (a) APP, its derivative or fragment thereof, (b) Myelination, (c) Sodium channel(s) and/or (d) stem cell(s).

In particular, the present invention provides the use of drugs, peptides or other molecules interacting with sodium channel(s) to modulate APP activity (function). The modulation of APP function may be used to prevent amyloid deposition (e.g. treatment of AD), to improve and/or facilitate nervous tissue repair (e.g. treatment of spinal injury), to improve and/or facilitate myelination (e.g. in the treatment of MS), or to modulate stem cell activity (function) (e.g. for brain repair). Examples of such drugs and molecules include but are not limited to local anaesthetics (e.g. lidocaine), veratridine, batrachotoxin, scorpion toxins, sea anaemone toxin, conus toxins, saxitoxin, tetrodotoxin, antiepileptic drugs (e.g. carbamazepine and phenytoin), antiarrhythmic drugs (e.g. quinidine), antibodies to sodium channel proteins, and peptide sequences derived from APP.

There is also provided the use of APP or peptides derived from the APP sequence to modulate sodium channel function. The modulation of sodium channel activity (function) may include modulation of APP expression and/or activity (function), treatment of acute or chronic pain disorders, induction of analgesia, treatment of epilepsy or convulsions, treatment of disorder of the rhythm of the heart, or induction of local anaesthesia.
There is also provided the use of drugs, peptides or other molecules interacting with TAG-1 to modulate APP function. The modulation of APP activity (function) may either be used to prevent amyloid deposition (e.g. treatment of AD), to improve and/or facilitate nervous tissue repair (e.g. treatment of spinal injury), to improve and/or facilitate myelination (e.g. in the treatment of MS), to modulate stem cell function (e.g. for brain repair), or to modulate sodium channel activity (function) (e.g. treatment of epilepsy or induction of local anaesthesia). Examples of such molecules include but are not limited to antibodies to TAG-1 and peptides derived from APP or Caspr.

There is also provided the use of TAG-1 or peptides which mimic part of the sequence of TAG-1 to modulate APP activity (function). The modulation of APP activity (function) may either be used to prevent amyloid deposition (e.g. treatment of AD), to facilitate nervous tissue repair (e.g. treatment of spinal injury), to improve and/or facilitate myelination (e.g. in the treatment of MS), to modulate stem cell function (e.g. for brain repair), or to modulate sodium channel activity (function) (e.g. treatment of epilepsy or induction of local anaesthesia). Examples of such molecules include but are not limited to full length TAG-1 and peptides comprising subset of the TAG-1 protein sequence.

There is also provided the use of drugs, peptides or other molecules interacting with TAG-1 or sodium channel peptide complex proteins to modulate APP function. The modulation of APP activity (function) may either be used to prevent amyloid deposition (e.g. treatment of AD), to improve and/or facilitate nervous tissue repair (e.g. treatment of spinal injury), to improve and/or facilitate myelination (e.g. in the treatment of MS), to modulate stem cell function (e.g. for brain repair), or to modulate sodium channel activity (function) (e.g. treatment of epilepsy and/or induction of local anaesthesia). TAG-1 or sodium channel peptide complex proteins include but are not limited to Caspr2, NgR, K+ channels, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ and
NG2. Examples of drugs, peptides or other molecules include but are not limited to peptide fragments of or antibodies to Caspr, NgR, K+ channels, TN-R, TNB-C, MAG, Nogo, phosphacan, F3, OMgp, RPTPβ and NG2.

The present invention also provides a method of detecting and/or quantitating the presence of, predisposition to, and/or severity of, a neurological condition in a subject, the method comprising: (a) providing at least one sample from a subject; (b) determining the expression and/or activity of amyloid precursor protein, its derivative or a fragment thereof; (c) comparing the expression and/or activity of the amyloid precursor protein, its derivative or a fragment thereof, with that of at least one control, a difference in expression and/or activity indicating the presence of, or predisposition to, and/or severity of a neurological condition in the subject.

The present invention also provides a method of monitoring the efficacy of a treatment for a neurological condition in a subject, the method comprising: (a) providing at least two samples from a subject, each sample obtained at different time points; (b) determining the expression or activity of amyloid precursor protein, its derivative or a fragment thereof; and (c) comparing the expression and/or activity of the amyloid precursor protein, its derivative or a fragment thereof, in the at least two samples.

The present invention also provides a method of prognosticating the outcome of a neurological condition in a subject, the method comprising: (a) providing at least one sample from a subject; (b) determining the expression and/or activity of amyloid precursor protein, its derivative or a fragment thereof; (c) comparing the expression and/or activity of the amyloid precursor protein, its derivative or a fragment thereof, with that of at least one control, a difference in expression and/or activity indicating the prognosis of a neurological condition in the subject.
The present invention also provides a method of inducing analgesia and/or anesthesia in a subject, the method comprising modulating the expression and/or activity of amyloid precursor protein, its derivative or a fragment thereof.

The present invention also provides a method of modulating differentiation of at least one stem cell, the method comprising decreasing expression and/or activity of amyloid precursor protein, its derivative or a fragment thereof, in the at least one stem cell. The decreasing may comprise administering an effective amount of a compound selected from the group consisting of TAG-1, anesthetics, toxins and Go protein(s).

There is also provided the use of conduction velocity measures as a marker for APP-sodium channel interactions and hence as a biomarker for APP malfunction and propensity for Alzheimer's disease or other amyloid-related dementia. In particular, there is also provided a method of detecting and/or quantitating the presence of, predisposition to, and/or severity of, at least one condition related to beta amyloid (Aβ) deposition and/or sodium channel dysfunction in a subject, the method comprising: (a) measuring the nerve and/or axon conduction velocity in the subject; (b) comparing the nerve and/or axon conduction velocity with that of at least one control, a difference in velocity indicating the presence of, or predisposition to, and/or severity of at least one condition related to Aβ deposition and/or sodium channel dysfunction in the subject. The method may be for detecting and/or quantitating the presence of, predisposition to, and/or severity of Alzheimer's disease (AD).

The present invention also provides at least one pharmaceutical composition for treating at least one APP and/or sodium channel related medical condition comprising at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one
sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s) toxin(s) and Go protein(s); and optionally at least one pharmaceutically acceptable diluent, carrier and/or excipient. The medical condition may be selected from the group consisting of Alzheimer’s disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders and demyelination disorders.

The present invention also provides use of at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s) toxin(s) and Go protein(s), for the preparation of a medicament (or pharmaceutical composition) for the treatment of an APP and/or sodium channel related medical condition.

The pharmaceutical composition may further comprise at least one pharmaceutically acceptable carrier, diluent, adjuvant, excipients, or a combination thereof. Examples of suitable excipients are water, saline, dextrose, glycerol, ethanol and the like as well as combinations thereof. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or alternatively the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carrier, excipient and/or diluent. Excipients normally employed for such formulations, includes mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. Further carrier, diluent, adjuvant, excipient or a combination thereof known in the art may also be used for the purpose of the present invention.
The pharmaceutical composition may be for local, subcutaneous, intravenous, parenteral and/or oral administration. The pharmaceutical composition may be administered through subcutaneous and/or intramuscular injection. For oral administration, the pharmaceutical composition may be formulated as solutions, suspensions, emulsions, tablets, pills, capsules, sustained release formulations, aerosols, powders, or granulates. The dosage required depends on the choice of the route of administration; the nature of the formulation; the nature of the subject's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Wide variations in the needed dosage are to be expected in view of the variety of compounds available and the different efficiencies of various routes of administration.

The present invention also provides a kit comprising at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s) toxin(s) and Go protein(s), for the detection and/or diagnosis of at least one APP and/or sodium channel related medical condition and/or for the detection and/or diagnosis of at least one APP and/or sodium channel related medical condition and/or treating and/or preventing at least one APP and/or sodium channel related medical condition.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.
EXAMPLES

Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Russel, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (2001).

Materials and Methods for Examples 1-10.

Antibodies
Rabbit polyclonal antibodies against Caspr (Nie et al, 2003) and monoclonal antibodies against TN-R (596, 619) (Xiao et al, 1998) and APP (Selkoe et al, 1988) were described previously. The antibodies recognise specifically APP intracellular domain (Selkoe et al., 1988). Rabbit polyclonal anti-Nav1.6, Navβ₂, pan-Nav (Alomone laboratories, Israel), monoclonal antibodies against pan-Na⁺ channel (K58/35; Sigma), potassium channels Kv1.2 (K14/16) (Upstate, USA), and γ-tubulin (GTU88; Sigma) were obtained from the respective commercial sources.

Mutant mice
The APP knock-out (Zheng et al, 1995), APP transgenic (Hsiao et al, 1995) and PLP transgenic (Inoue et al, 1996) mice have been previously described. APP knock-out mice exhibit decreased locomotor activity and forelimb grip strength (Zheng et al., 1995), consistent with myelinated axon-related abnormalities. APP transgenic mice over-express human APP driven by the prion promoter. APP protein expression in these transgenic mice depends upon copy number (Hsiao KK, et al., 1995). The PLP transgenic mouse (PLP tg; Inoue et al., 1996; Rasband et al., 2003) is an animal model of severe demyelination.

All experiments involving mice were approved by the Institutional Animal Care and Use Committee (IACUC) of Singapore General Hospital. The experiments
involving *Xenopus laevis* oocytes were approved by the IACUC of the National University of Singapore.

**Immunofluorescence (IF) labelling**

Animals were perfused transcardially with 0.1 M Ringer's solution and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB) sequentially. Spinal cords were removed immediately for different subsequent preparations. For cryosection, tissues were immersed, first in 15% sucrose and then in 30% sucrose in PB. Ten micrometer-thick sections were mounted onto gelatin or poly-l-lysine coated glass slides. After being air-dried, slides were kept at -20°C for later use.

For immunofluorescence staining, tissue slices were first immersed in absolute acetone at -20°C for 20 min. Slices were then thoroughly washed in 0.1 M phosphate buffered saline (PBS, pH 7.6) containing 0.3% Triton X-100. After blocking with 10% normal goat serum (NGS) diluted in PB for 1 hr, tissue slices were incubated with primary antibodies at their optimum dilutions, either overnight or 2 hrs at room temperature (RT). Cy2- or Cy3-conjugated secondary antibodies (Amersham) corresponding to the primary antibodies were used for further incubation at RT for 1 hr. For double labelling, one more cycle of labelling was similarly conducted for second primary antibodies. All labeled slices were visualized and photographed under a Leica RXA2 upright fluorescence microscope or an LSM5 Carl Zeiss confocal microscopic system.

**Western blot analysis**

To prepare whole extracts, frozen tissues (spinal cords and brains) were homogenized in a lysis buffer (50 mM Tris-HCl pH 7.5, 5 mM EDTA, 1% Triton X-100, and protease inhibitors (Roche Diagnostics). After ultracentrifugation (150,000 g, 4°C, 45 min), the supernatants were collected and stored at -80°C for future use. Alternatively, membrane fractions were prepared as previously
described (Nie et al, 2003). Brain tissue or cells were homogenized in ice-cold homogenizing buffer (320 mM sucrose, 10 mM Tris-HCl pH 7.4, 1 mM NaHCO₃ pH 7.4, 1 mM MgCb) supplemented with 1% protease inhibitor cocktail (Amersham), and subsequently centrifuged at 5,000 g for 15 min. The supernatant was collected and spun at 60,000 g (Beckman ultracentrifuge) for 60 min at 4°C. Pellets were then dissolved in a lysis buffer (10 mM Tris-HCl pH9, 150 mM NaCl, 0.5% Triton X-100, 1% sodium deoxycholate (DOC), 0.5% SDS, 2 mM EDTA, and 1% protease inhibitor cocktail. All samples were subjected to Bradford protein assay.

Equal amounts of protein were separated on acrylamide gels and transferred onto nitrocellulose membranes. Western blotting was performed under standard conditions, applying rabbit polyclonal antibodies against APP (1:1000) and mouse monoclonal antibodies against γ-tubulin (1:1000). The latter protein was used for loading normalization. Either anti-mouse, or anti-rabbit peroxidase-conjugated secondary antibodies were applied at 1:10,000 and blots were visualized with an ECL™ detection kit (Amersham).

Electromicroscopy

Animals were transcardially perfusion-fixed with 2% paraformaldehyde - 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for electron microscopy. Spinal cords and sciatic nerves were removed and post-fixed in the same fixative solution for 2 hrs. After being finely trimmed, the samples were immersion-washed overnight. Tissue blocks were further post-fixed in 1% osmium tetroxide (OsO₄) containing 1.5% potassium ferrocyanide for 2 hrs, and thereafter subjected to dehydration in ascending series of alcohol and acetone. After undergoing gradual infiltration with Araldite 502 (EMS), the blocks were embedded and polymerized overnight at 60°C. Ultrathin sections (~90 nm in thickness) were placed on 200-mesh copper grids and counterstained with uranyl acetate and lead citrate. All samples were examined and photographed.
under a Jeol 1220 electron microscope. The \( g \) ratios were calculated from more than 50 myelinated axons per mouse (\( n = 3 \)).

**Plasmid construction and cRNA transcription**

The vector pD4GI\(_{-}\), a modified version of the pcDNA4A vector (Invitrogen) that contains the *Xenopus* \( \beta \)-globin untranslated regions, was used to boost expression in oocytes. Here, the *Xenopus* \( \beta \)-globin 5' and 3' untranslated regions were subcloned into the pcDNA4A vector. cDNAs encoding the rat \( \beta_1 \) and \( \beta_2 \) subunits (provided by Lori Isom, University of Michigan), the AICD domain (SEQ ID NO: 4 cDNA; SEQ ID NO: 7, peptide), C-terminal fragment C31 domain (SEQ ID NO: 8, cDNA; SEQ ID NO: 10, peptide), G protein Go binding domain (SEQ ID NO: 11; SEQ ID NO: 14, peptide), Fe65 binding domain (SEQ ID NO: 15, cDNA; SEQ ID NO: 18, peptide) and a short peptide motif Val667-Arg672 (SEQ ID NO: 19 cDNA; SEQ ID NO: 22, peptide) were inserted between these two regions. To facilitate translational recognition by ribosomes, a Kozak sequence was added to around the start codon (GCCACCATGG) (SEQ ID NO: 1) for oocyte expression. The pLCT1-Scn8a plasmid containing mouse Nav1.6 cDNA was provided by Dr. Alan L. Goldin (University of California, Irvine). The plasmid containing cDNA of Nav1.5 \( \alpha \) subunit of sodium channels was provided by Dr. A. L. George (Vanderbilt University, Nashville). The plasmid encoding the human App695 cDNA was provided by Dr. Carsten Schmidt (University of Hamburg).

The AICD domain (forward primer: 5'-TTTGGTG AAGCTT GCCACCATG GTG ATG CTG AAG AAG AAA CAG TAC-3' (SEQ ID NO: 5); reverse primer: 5'-GGCG GCGGCCGC CTA GTT CTG CAT CTG CTC AAA GA-3' (SEQ ID NO: 6) G protein Go binding domain (forward primer: 5'-GCAG AAGCTT GCCACC ATG CATCATGGTGTGG TGGAGGTTG-3' (SEQ ID NO: 12); reverse primer: 5'-GGCG GCGGCCGC CTA CTT GGA CAG GTG GCG CTC CTC-3'(SEQ ID
NO: 13), C-terminal C31 domain (forward primer: 5'-GCGC AAGCTT GCCACC ATG GCCGCTGTCACCCCAGAGGAG-3' (SEQ ID NO: 9); reverse primer: 5'GGCG GCGGCCGC CTA GTTCTGCATCTGCTCAAAGA-3'(SEQ ID NO: 6), Fe65 binding domain (forward primer: 5'-GCGC AAGCTT GCCACC ATG GTCACCCCAGAGGAGCGCCAC-3'(SEQ ID NO: 16); reverse primer: 5'GGCG GCGGCCGC CTAGTTGTAGGTTGGATTTTCGT-3'(SEQ ID NO: 17), Val667-Arg672 motif (forward primer: 5'-AGCTT GCCACC ATG GTC ACC CCA GAG GAG CGC TAG GC-3'(SEQ ID NO: 19); reverse primer: 5'GGCCGC CTA GCG CTC CTC TGG GGT GAC CAT GGTGGC A-3'(SEQ ID NO: 20) were obtained from the full length human APP-695(SEQ ID NO: 2).

Plasmids were purified using QIAGEN Plasmid Maxi Kit (QIAGEN). All these purified constructs were linearized, and cRNAs were synthesized in vitro using the mMESSAGE mMACHINETM T7 cRNA synthesis kit (Ambion). The concentration of cRNA was determined from OD (260 nm).

Expression and electrophysiological recording in oocytes
Stage V oocytes were surgically removed from mature female *Xenopus laevis* (Xenopus Express, France) anaesthetized with 1 g/L tricane methanesulphonate (Sigma, USA). Segments of ovarian lobe were removed by a small abdominal incision. The follicular layer was removed by digestion for 90 min with 2 mg/ml collagenase type V (Sigma, USA) in Ca²⁺-free Barth's solution containing (mmol/L) NaCl 88, KCl 1, NaHCO₃ 2.4, MgSO₄ 0.82, Tris/HCl 5; pH 7.4 with NaOH; supplemented with 50 µg/ml gentamicin sulphonate). The oocytes were placed in 96-well plates and incubated at 19°C in Barth's solution overnight. On the following day oocytes were injected (Roboocyte, Multichannel Systems, Germany) with cRNAs. RNA was dissolved in 1 mM Tris-HCl, pH 7.5. The molar ratio of α to β subunits was 1:10, while that of the α subunit to APP was 1:1. The oocytes were then incubated at 19°C. Membrane currents were recorded from oocytes with the two-microelectrode voltage-clamp technique at
room temperature (19°C to 21°C) 2 to 3 days after injection of cRNA. Both injection and recording were performed using Roboocyte (Multi Channel Systems, Germany). Current injecting and potential measuring electrodes had resistances of 0.1 to 0.7 MΩ when filled with 3 mol/L KCl. Data acquisition was performed with a GeneClamp 500B two-electrode voltage clamp amplifier connected to a Digidata 1322A interface and controlled with pClamp 9.2 software (all from Axon Instruments, Inc., Foster City, CA). Oocytes were perfused with ND96 solution containing (mmol/L) NaCl 96, KCl 2, CaCl₂ 1.8, MgCl₂ 1, and HEPES 5 (adjusted to pH 7.4 with NaOH). Oocytes were kept in current-clamp mode for at least 2 minutes before switching to voltage-clamp mode. All recordings were obtained after stable baseline and ionic current levels were achieved. Data was sampled at 50 kHz after filtering at 5 kHz with a 4-pole Bessel filter. The leakage current was digitally subtracted on-line using hyperpolarizing control pulses, applied before the test pulse, of one-fourth-test pulse amplitude (-P/4 procedure).

Oocytes were held at -100 mV and 45 ms steps to command potentials between -75 mV and 40 mV were made in 5 mV increments to assess the current-voltage (I-V) relationship. Activation was assessed by calculating conductance using the equation \( G = \frac{1}{I(V-E_{Na})} \) (where I is the peak current amplitude at command potential V and \( E_{Na} \) is the equilibrium potential for Na⁺ under our experimental conditions) and fitting the data to a Boltzmann distribution function: \( G_{Na} / G_{Na,\text{max}} = \frac{1}{1+\exp(V_{1/2} - V_m)/k} \), where \( G_{Na} \) is the voltage-dependent sodium conductance, \( G_{Na,\text{max}} \) is the maximal sodium conductance, \( V_{1/2} \) is the potential at which activation is half-maximal, \( V_m \) is the membrane potential, and k is the slope. For statistical analysis, the peak current amplitude was recorded for each oocyte. Inactivation was assessed with a series of pre-pulses (-120 to 10 mV) lasting 500 ms from the holding potential of -100 mV, followed by a 25 ms depolarization to -10 mV. The normalized curves were fitted using a Boltzmann distribution equation \( (G_{Na} / G_{Na,\text{max}})^{1/1+\exp(V_m - } \)
Recovery from inactivation was assessed with two 50 ms pulses to -10 mV from the holding potential of -100 mV, with a variable recovery time period at -100 mV, in the range of 1 to 30 ms. Curves were fitted with a single rising exponential function.

All data are expressed as mean ± SEM. Student's t test was used to test for statistical significance of effects on peak current amplitude and $V_{1/2}$. Two-way ANOVA for effects of APP and recovery time was used to analyze the data on recovery from inactivation. A value of $p<0.05$ was considered significant.

Electrophysiological recordings of dorsal root ganglion neurons

Acutely isolated dorsal root ganglion (DRG) neurons were prepared from the sensorimotor cortex of anesthetized and then decapitated adult APP $^{+/-}$ and $APP^{+}C57BL$ mice. The lumbar segments of vertebrate column were dissected. The DRGs, together with the nerve roots, were quickly removed and transferred immediately into Dulbecco's modified Eagle's Medium (DMEM, Gibco, Carlsbad, CA). After removal of attached nerves and surrounding connective tissues, the DRGs were minced with fine spring scissors and the ganglion fragments were placed in a flask containing 1.5 ml of DMEM in which trypsin (type III, 0.5 mg/ml, Sigma, St. Louis, MO), collagenase (type I, 1 mg/ml, Sigma) and DNase (type I, 0.1 mg/ml, Sigma) had been dissolved. After incubation at 35°C in a shaking water bath for 30 min, soybean trypsin inhibitor (type II-s, 1.25 mg/ml, Sigma) was then added to stop trypsin digestion. DRGs were carefully removed by Pasteur pipette and placed in bath solution containing (in mM): 140 NaCl, 3 KCl, 1 MgCl$_2$, 1 CaCl$_2$, 0.1 CdCl$_2$, and 20 HEPES, pH 7.3 adjusted to 320 mosmol/l with glucose. The DRGs were mechanically dispersed (5 strokes up and down) with a 1-ml pipette and plated onto a 35-mm culture dish and kept for at least 30 min before electrophysiological recordings. All recordings were made at room temperature (20-24°C) within 10 h after dissociation to keep the experiment as similar to in vivo as possible.
Current signals from acutely isolated DRG neurons, recorded in whole cell voltage clamp mode, were sampled at 20 kHz and filtered at 5 kHz using a MultiClamp 700A amplifier in conjunction with a Digidata 1322A interface and pClamp 8.1 software (all from Axon Instruments, Inc., Foster City, CA). Micropipettes were pulled from borosilicate glasses (Boralex) with a Flaming Brown micropipette puller (P2000, Sutter Instrument, Novato, CA) an electrode resistance ranging from 2 to 5 MΩ. The pipette solution contained (in mM) 140 CsF, 1 EGTA, 10 NaCl, and 10 HEPES, pH 7.3, and was adjusted to 310 mosmol/l with glucose. CdCb in bath solution was used to block Ca²⁺ currents. To eliminate the small residual sustained outward current that was seen at very depolarizing pulses, in some experiments we included 10 and 20 mM TEA in bath and pipette solutions, respectively, and CsF concentration was decreased to 120 mM in the pipette solution. The pipette potential was zeroed before seal formation, and the voltages were not corrected for liquid junction potential. Capacity transients were cancelled, and series resistance was compensated (75-80%) as necessary. The leakage current was digitally subtracted on-line using hyperpolarizing control pulses, applied before the test pulse, of one-fourth test pulse amplitude (P/4 procedure). Data are presented as means ± S.E and statistical analyses were performed using the Student's t test (significance at least p < 0.05).

Voltage protocols were as follows. Standard current-voltage (I-V) families were obtained using 50 msec pulses from a holding potential of -110 mV to a range of potentials (-60 to 50 mV) in 10-mV increments. The peak value at each potential was plotted to form I-V curves. Activation curves were fitted with the following Boltzmann distribution equation: \[ \frac{G_{Na}}{G_{Na,max}} = \frac{1}{1 + \exp(V_{1/2} - V_m)/k} \]
where \( G_{Na} \) is the voltage-dependent sodium conductance, \( G_{Na,max} \) is the maximal sodium conductance, \( V_{1/2} \) is the potential at which activation is half-maximal, \( V_m \) is the membrane potential, and \( k \) is the slope. Availability protocols consisted of a series of pre-pulses (-100 to 10mV) lasting 100 ms from the
holding potential of -70 mV, followed by a 100 ms depolarization to -10 mV. The normalized curves were fitted using a Boltzmann distribution equation:

\[ I_{Na} / I_{Na,m} = 1 /[1 + \exp((V_m - V_{1/2})/k)] \]

where \( I_{Na,m} \) is the peak sodium current elicited after the most hyperpolarized prepulse, \( V_m \) is the preconditioning pulse potential, \( V_{1/2} \) is the half-maximal sodium current, and \( k \) is the slope factor. For recovery from inactivation experiments, two 40 ms stimuli were given to -10 mV from the holding potential of -110 mV, with a variable recovery time period at -110 mV, in the range of 1 to 400 ms. Curves were fitted with a double rising exponential function.

Conduction velocity recording

Electrophysiology for spinal cord

Conduction properties of myelinated axons were examined in acutely isolated spinal cords of C57BL/6 mice, using a double grease-gap technique (Coetzee et al, 1996). The spinal cord was placed in a Plexiglas chamber, with the central channel (15 mm wide) superfused by flowing, oxygenated Krebs' solution and the two ends isolated by grease gaps in chambers containing isotonic (120 mM) potassium chloride solution. Silver/silver chloride electrodes were used to record the potential across one gap and to stimulate the spinal cord across the other. This provided a stable recording and stimulating arrangement with a fixed conduction distance of 15 mm for the spinal cord. The animals were anesthetized, and then decapitated and the spinal cord extracted by rapid laminectomy and washed in oxygenated Krebs' solution (NaCl 119 mM, KCl 2.5 mM, NaH₂PO₄ 1 mM, MgSO₄ 1.3 mM, CaCl₂ 2.5 mM, D-glucose 11 mM, NaHCO₃ 26.2 mM, equilibrated with 95% O₂, 5%CO₂). The cord was incubated in Krebs' solution at room temperature for at least 1 hr, and then mounted in a recording chamber. The temperature of chamber was raised to 32°C and 37°C, and a series of recordings were made to stimulation that was adjusted to 10%
above the level that elicited a maximum response. The data were digitized at 20 kHz for subsequent analysis.

**Electrophysiology for sciatic nerve**

Sciatic nerves were dissected immediately after animals were killed and placed in oxygenated Krebs' solution. Nerves were then transferred to a recording chamber that was continuously perfused with oxygenated Krebs' solution. Stimulation was delivered onto one end of nerve by a concentric bipolar electrodes (Frederick Haer Co., Bowdoinham, ME, USA) and the other end of the nerve was drawn into a suction electrode for recording action potentials (Stys et al, 1991). Compound action potential of nerve recorded by suction electrode (Weber, 1999).

**Electrophysiological recordings of Nav1.6 cell line**

Whole-cell voltage-clamp recordings were performed in a human embryonic kidney 293-cell line stably expressing human Nav1.6 (Oliveira et al., 2004) using a Port-a-Patch NPC1 (Nanion Technologies GmbH, Munich, Germany) and Multiclamp700A amplifier (Axon Instruments (Molecular Devices), CA, USA). The external, internal and seal enhancer solutions were as supplied by Nanion. The VTPEER peptide was synthesised (Anaspec) and dissolved in internal solution. Capacity transients were cancelled, and series resistance was compensated by 85-90%. Leakage current was digitally subtracted on-line using a hyperpolarizing potential supplied after the test pulse (P/4 procedure). Currents were acquired at a sampling rate of 20 kHz via a Digidata 1322A series interface (Axon Instruments (Molecular Devices), CA, USA) controlled by pClamp 9.2 software (Axon Instruments (Molecular Devices), CA, USA) and filtered at 5 kHz. All experiments were performed at room temperature (21-24°C).
Voltage protocols were implemented at predetermined intervals after going whole cell. Briefly, standard current-voltage (I-V) families were obtained using 40ms pulses from a holding potential of -120mV, to a range of potentials (-65 to -60mV) every 5s. The peak value was plotted to form I-V curves. To plot I-V relationship, the currents at each potential were normalized to pre-treatment peak current. Activation curves were fitted with the following Boltzmann distribution equation: 

\[ G_{Na} = \frac{G_{Na,max}}{1 + \exp \left( \frac{V_{m} - V_{1/2}}{k} \right)} \]

where \( G_{Na} \) is the voltage-dependent sodium conductance, \( G_{Na,max} \) is the maximal sodium conductance, \( V_{1/2} \) is the potential at which activation is half-maximal, \( V_{m} \) is the membrane potential, and \( k \) is the slope. Availability protocols consisted of a series of prepulses (-120 to +2OmV) lasting 500ms, from the holding potential of -90mV, followed by a 40ms depolarization to -10mV, every 10s. The normalized curves were fitted using a Boltzmann distribution equation: 

\[ \frac{I_{Na}}{I_{Na,max}} = \frac{1}{1 + \exp \left( \frac{V_{m} - V_{1/2}}{k} \right)} \]

where \( I_{Na,max} \) is the peak sodium current elicited after the most hyperpolarized prepulse, \( V_{m} \) is the preconditioning pulse potential, \( V_{1/2} \) is the half-maximal sodium current, and \( k \) is the slope factor. For recovery from inactivation experiments, two 40 ms stimuli were given to -10 mV from the holding potential of -120 mV, with a variable recovery time period at -120 mV; in the range of 1 to 400 ms. Curves were fitted with a double rising exponential function.

**Example 1 - Distribution of APP**

Myelinated axons in the CNS are characterized by their segmental structures: the node of Ranvier (NOR), paranode, juxtaparanode, and internode. We used an antibody against the APP intracellular domain to (Selkoe et al, 1988) to investigate the distribution of APP along myelinated axons of both CNS and PNS. In longitudinal sections of the ventral part of adult rat spinal cord, APP immunoreactivity (green) was confined specifically to NORs along myelinated axons (Fig. 4). As evidenced by double immunofluorescence (IF) labelling, the
APP staining (green) was flanked by juxtaparanodal K+ channels Kv1.214 (Kch; red; Fig. 4a, d, e, f, and g) and co-localized with Tn-R6 (red; Fig. 4b, h, i, j, and k) and Na+ channels (Nach; red; Fig. 4c), two specific nodal molecules. Similar nodal localization of APP was also observed in optic nerve and other brain areas (not shown). The specific labelling of APP in NORs was undetectable after the antibody was pre-incubated with APP protein. The number of nodes labelling for APP was quantified relative to labelling with an antibody to the axonal marker, Kv1.2. The ratio of APP to Kv1.2, reflecting the portion of APP in all NORs, was above 95% (Fig. 8), implying that APP is located at almost every NOR in the CNS. As the APP antibody applied for the IF staining recognizes the intracellular domain and the axon is exposed at the NORs in the CNS, these observations indicate that the clustered nodal APP is most likely mainly in axons. APP is also expressed in the PNS (Golde et al., 1990). However, in contrast to APP’s nodal location in the CNS, APP immunoreactivity (green) was evenly distributed (Fig. 5) along adult rat sciatic nerve. This was shown by double IF labelling with NF200 (red; Fig. 5a), an axonal marker, or juxtaparanodal K+ channels Kv1.2 (red; Fig. 5b). Moreover, the nodal location of APP in the CNS was further investigated in longitudinal sections of the ventral part of spinal cords from APP knock-out mice by double IF labelling of Tn-R and APP or Caspr, a specific paranodal molecule, or Caspr and Nach (sodium channels). At NORs, APP clusters were absent in sections from APP knock-out mice (Fig. 5c). However, Tn-R, Nach, and Caspr clusters remained in the mutant mice (Fig. 5d and e). These experiments demonstrate that axonal APP is a novel component of NORs and its clustering occurs specifically in the CNS, but not in the PNS. Further, these observations demonstrate that nodal APP has no effects on the clustering of Tn-R, sodium channels and caspr in the nodal regions of the CNS.
Example 2 - distribution and expression profile of APP during development.

Myelination is a dynamic process mediated by bidirectional axoglial interactions that are involved in the clustering of axonal molecules, such as Na+ channels, F3/contactin, and OMgp, at NORs (Salzer 2003; Ma et al., 2007). We investigated the distribution and expression profile of APP during development, especially in relation to the advance of myelination and nodal formation. As illustrated by Western blotting analysis (Fig. 19A), APP was expressed in brain as early as at birth (PO); however, it was greatly up-regulated between postnatal day 5 (P5) to day 21 (P21). The time course of APP appearance parallels the time frame for myelination (Huber et al., 2002), suggesting a role for APP in myelination. Moreover, IF studies were performed to correlate aggregation of APP with Na+ and K+ channel clustering during nodal maturation. At P6, when Na+ channels start clustering at NORs, APP was diffusely distributed along axons (Fig. 6a). APP was distributed along axons and appeared to start clustering in the NORs around P10 (Fig. 6b) and was prevalent at nodes after P15, when the nodal structure has been formed (Fig. 6c and d). These observations indicate that APP clustering lags behind the Na+ channel clustering in the CNS. The intensity of APP nodal clustering was quantified in relation to Kv1.2 during development. The ratio of APP to Kv1.2, reflecting the developmental progress of APP clustering in the population of axons, was 50% at P10, 60% at P15 and P21, and 90% at 2 months (Fig. 7), implying that APP clustering also occurs latter than the K+ channel clustering in the CNS.

Example 3 - nodal accumulation of APP relies on the integrity of distinct axonal domains.

APP has long been utilized as a marker for axonal degeneration following neural injury (Kuhlmann et al., 2002). In the spinal cord of EAE mice, APP co-localizes extensively with Nav1.6 and to a lesser extent with Nav1.2 sodium
channels at sites of axonal injury. This is evidenced by diffuse distribution of both APP, Nav 1.6 and, to a lesser extent, Nav1.2 along demyelinated axons and an increase in the number of diffusely APP-positive axons (Craner et al, 2004). These observations suggest a spatial relationship between APP and these two nodal channels in this pathological condition. IF was performed using the spinal cord of 7-month-old PLP transgenic mice (PLP tg) (Rasband, 2003; Inoue et al., 1996), an animal model of severe demyelination. In PLP tg mice, double labelling of K+ channels (red) or the 200 kDa neurofilament (red), two axonal markers, with APP (green) revealed that APP clustering completely disappeared in demyelinated axons (Fig. 19B). Given that APP clustering along axons is severely disrupted in PLP tg mice as well as in the EAE model, these results support the notion that nodal accumulation of APP relies on the integrity of distinct axonal domains. Together, the above observations in both physiological and pathological animal models demonstrate that, similar to the clustering of Na+ channels and other axonal molecules at NORs, APP nodal aggregation along CNS myelinated axons requires axoglial interactions.

Example 4 - APP regulates axonal size and myelin thickness

APP knock-out mice exhibit decreased locomotor activity and forelimb grip strength (Zheng et al, 1995), consistent with myelinated axon-related abnormalities. Given the nodal location of APP and its expression pattern in the CNS during myelination, we analyzed the spinal cord from 3-month-old APP knock-out mice by electron microscopy (EM) to investigate that APP plays a role in the development of myelinated axons. No axonal swelling was found in mutant mice at this age (Fig. 9b). Both axon and fiber diameters were significantly reduced in the spinal cord of the knock-out mice versus wild-type mice (Fig. 20a and b; Fig. 9c). However, the g ratio (the numeric ratio between the diameter of the axon and the outer diameter of the myelinated fiber (Michailov, 2004) was significantly increased in the spinal cord of the knock-out
mice (0.829 ± 0.002) in comparison to wild-type mice (0.817 ± 0.002) (Fig. 9c; p < 0.001). To thoroughly characterize the role of APP in determining myelin thickness, we grouped the myelinated fibers by the axon diameter (<1.0 µm; 1.0-1.5 µm; 1.5-2.0 µm; 2.0-2.5 µm; 2.5-3.0 µm; 3.0-4.0 µm; and >4.0 µm).

The g ratio showed that the hypomyelination in APP knockout mice was significant in all axons (Fig. 20c). These observations suggest that, as an axonal molecule, APP not only regulates axonal size, but also myelin thickness.

Moreover, the degree of peripheral myelination in the sciatic nerve from APP wild-type and knock-out mice was analysed by using EM. In wild-type mice, the average g ratio was 0.715 ± 0.002 whereas myelin in APP--/ mice was significantly thinner (p < 0.001) with a g ratio of 0.754 ± 0.038 (Fig. 10), indicating a thinner myelin sheath encapsulating the axons. The analysis of g ratios across all axon sizes was done. The reduction in the myelin sheaths occurred in nearly all axons, as illustrated by increased g ratios in axons of all sizes, but was more significant in small to medium sized axons (2.0 µm to 7.0 µm; p < 0.01; Fig. 21). Thus, APP plays a common role in promoting myelination in both CNS and PNS.

Example 5 - APP plays a role in myelination of axons
We analyzed spinal cords from 3-month-old transgenic mice that overexpress human APP. Driven by the prion promoter, APP protein expression in these transgenic mice was previously reported to depend upon copy number (Hsiao et al, 1995). No axonal swelling was found in these mutant mice at this age (Fig. 11b). Interestingly, both axon and fiber diameters were significantly increased (FIG. 22a and b), while the g ratio was significantly decreased in the transgenic mice (Fig. 11c) compared to wild-type mice. Although the APP knock-out and transgenic over expression mice have different strain backgrounds (C57BL/6 and FVB/N, respectively), the reversible changes in axon and fiber diameters
and myelin thickness between these two mutants have been confirmed after normalization with their respective wild-type littermates (Fig. 12a, b, and c). Together, these data demonstrate that APP plays a positive role in the development of myelinated axons.

Example 6 - APP interacts with sodium channels

Voltage-gated Na+ channels consist of a pore-forming α subunit and at least one β subunit that regulates channel behavior and surface expression (Catterall, 2000). Among the nine functionally expressed members of the α subunit family, Nav1.6 produces both transient and persistent currents (Rush et al, 2005) and localizes predominantly at adult NORs, but not in the intermodal region of myelinated axons (Caldwell, 2000). Na+ channel β1 and β2 subunits also localize at NORs (Chen et al, 2004).

The inventors coexpressed Nav1.6 with full length APP in various molar ratios in *Xenopus laevis* oocytes to investigate whether APP could act as a β subunit of sodium channels. Notably, the increase in the sodium current in the presence of APP was not in a dose dependent manner, indicating that APP does not act as a β subunit of sodium channels (Fig. 28).

Sodium channels β1 and β2 were previously reported to localize at NORs (McEwen and Isom, 2004). To investigate these interactions of APP with Nav1.6 and the roles of β subunits in regulation of APP dependent sodium currents, the inventors co-expressed full length APP with the Nav1.6 α subunit alone or together with the β1 and β2 subunits either individually or in combination in *Xenopus laevis* oocytes. The inventors also co-expressed APP with Nav1.5 α subunit. Na+ currents were activated by stepped depolarisations. Greater currents were obtained in the presence of APP than in the absence of APP for all subunit combinations (Fig. 13a and b, 14a and b, 15a and b, and 16a and b). Greater currents were obtained for Nav1.6 in the presence of APP...
(Fig. 13b), but not after control injection of cRNA for the reverse sequence (Fig. 13g) nor for Nav1.5 (Fig. 13f). Populations of oocytes were sampled from four frogs for each expression combination. To plot the current-voltage (I-V) relationship, the currents were normalised to the mean peak current in the absence of APP for each frog. The I-V curves confirmed that the currents were greater in the presence of APP than in the absence of APP (Fig. 13b, 14b, 15b, and 16b). For statistical analysis, the peak current in each oocyte was determined. APP increased the peak current 1.74 ± 0.11 fold on expression of the α subunit alone (FIG. 23a; p < 0.001), 2.38 ± 0.55 fold on expression of the α subunit and β1 subunit in combination (FIG. 23b; p < 0.001), 1.84 ± 0.21 fold on expression of the α subunit and β2 subunit in combination (FIG. 23c; p < 0.001), and 2.47 ± 0.22 fold on expression of the α subunit together with both β1 and β2 subunits (FIG. 23d; p < 0.001). The increase in the current in the presence of APP appeared to be greater when the β1 subunit was expressed as the sole β subunit (Fig. 14b, FIG. 23b) or in combination with the β2 subunit (Fig. 16b, FIG. 23d). Conductance was calculated from the I-V data and activation curves were fitted with a Boltzmann function to estimate V1/2, the potential at which activation is half-maximal (Fig. 13c, 14c, 15c, and 16c).

APP had no significant effect on activation when expressed together with the α subunit alone (Fig. 13c) or the α and β2 subunits (Fig. 15c), but shifted V1/2 to the left when the β1 subunit was expressed together with the α subunit (from -41.03 ± 2.3 in the absence of APP to -47.86 ± 1.60 in the presence of APP; p < 0.005; Fig. 14c) or together with the α and β2 subunits (from -42.80 ± 1.68 in the absence of APP to -51.67 ± 1.60 in the presence of APP; p <0.005; Fig. 16c).

Inactivation protocols consisted of a series of pre-pulses followed by a short depolarisation. The normalized curves were fitted using a Boltzmann distribution equation. The presence of APP had no significant effect on the Na+ inactivation currents (Fig. 13d, 14d, 15d, and 16d). For recovery from inactivation
experiments, two pulses were applied with a variable recovery time (1 to 30 ms) at a holding potential of -100 mV. Curves were fitted with an exponential function. The presence of APP had no significant effect on the recovery from inactivation currents in the presence of the α subunit alone (Fig. 13e) or together with the β1 subunit (Fig. 14e). However, APP accelerated recovery in the presence of the β2 subunit when expressed as the sole beta subunit (ANOVA APP effect, F1.1080 = 147.4, p < 0.001; Fig. 15e) or together with the β1 (ANOVA APP effect, F1.1200 = 115.1, p < 0.001; Fig. 16e).

These data demonstrate that APP specifically increased Nav1.6 currents in *Xenopus* oocytes whether the channel was expressed with the α subunit alone or with the β1 or β2 subunits present. APP appeared to cause a greater increase in Na+ currents and produced a shift in the activation current towards more negative potentials only in the presence of the β1 subunit, whether as the only β subunit or in combination with the β2 subunit. APP accelerated recovery from inactivation only in the presence of the β2 subunit, whether as the only β subunit or in combination with the β1 subunit. Together these data suggest that APP interacts both with the α subunit alone and with the β subunits.

**Example 7 - Whole cell recordings from DRG neurons**

Next, we performed whole cell recordings from DRG neurons that were acutely isolated from APP knockout and wild type mice. After pharmacological suppression of voltage-dependent Ca2+ and K+ currents with Cd2+ and Cs+, respectively, fast Na+ currents were activated by stepped depolarizations (Fig. 4Aa top). To determine the I-V relationship of the Na+ current, the peak current amplitude at each voltage step was normalized to the neuron's capacitance and plotted as a function of the command potential (Fig. 17a bottom). As capacitance is related to cell size, correction for capacitance in this manner should reflect changes that are attributable to difference in cell size in the knockout mice.
Na+ current densities were significantly decreased in DRG neurons from APP knockout mice versus wild-type mice (p < 0.05). The activation curve of the Na+ conductance was constructed from the current-voltage relationship. There was no difference in the activation of Na+ currents in the wild type and APP-deficient mice (p > 0.05; Fig. 17b). Steady-state inactivation of Na+ currents was determined. Current amplitudes were expressed as a fraction of the maximal current amplitude and plotted as a function of the prepulse potential. Steady-state inactivation did significantly differ between the wild-type and APP-deficient mice (Fig. 17c). Recovery from inactivation was studied by gradually increasing the interval (1 to 400ms) between two depolarising test pulses.

The peak amplitude of the second Na+ current response divided by the response at the maximal interval was then plotted as a function of the interpulse interval. As shown in Fig. 17d, there was no significant difference in recovery from inactivation between the two groups. Thus, these observations indicate that APP positively modulates Na+ current density in mammalian cells. The β subunit-dependent effects of APP on activation and recovery from inactivation suggested by the experiments in Xenopus oocytes were not replicated in the mammalian cells. This may be explained by differences in Nav1.6 regulation in Xenopus oocytes and mammalian DRG or by the presence of additional native Na+ channel subtypes in the mammalian DRG. Nevertheless, the observations on Na+ current amplitude in Xenopus oocytes and mammalian DRG are consistent and indicate that APP positively modulates Na+ currents. The effect of APP on Na+ currents is unlikely to be due to changes in cell size as, in the DRG recordings, changes were still seen in current densities corrected for whole-cell capacitance. The effect of APP on Na+ currents reflects either increased expression of Na+ channels in the cell membrane or increased conductance of individual Na+ channels in the presence of APP.
Example 8 - APP modulates CNS action potentials

The congregation of specific ion channels and cell recognition molecules, such as Na+ channels and F3/contactin, at NORs, is critical for the rapid saltatory conduction of nerve impulses. To further explore the physiological role of clustered APP in the CNS, we recorded compound action potentials of APP knock-out versus wild-type mice. We used a double grease-gap chamber (fixed conduction distance (Coetzee et al., 1996) and suction electrode (Kazarinova-Noyes, et al., 2001) to measure CAPs from spinal cord and sciatic nerve, respectively. The time-to-peak for each trace was then measured and converted to a conduction velocity.

The average velocity in wild-type mice was faster than those from the mutant mice at all temperatures measured. At 24°C, the conduction velocity of control spinal cord was $12.83 \pm 0.42$ ms$^{-1}$ ($n = 8$) and that of APP-deficient spinal cords was $11.34 \pm 0.49$ ms$^{-1}$ ($n = 11$) ($p < 0.05$; Fig. 18a). At 37°C, the conduction velocities were $22.69 \pm 0.10$ ms$^{-1}$ ($n = 8$) in wild-type mice and $18.53 \pm 0.77$ ms$^{-1}$ ($n = 11$) in APP-deficient mice ($p < 0.005$, Fig. 18a). Moreover, we tested sciatic nerves from APP knock-out and wild-type mice as a control using suction electrodes to measure CAPs from sciatic nerves.

The conduction velocities at 24°C in nerves from the wild-type mouse averaged $22.23 \pm 1.71$ ms$^{-1}$ ($n = 6$); in the APP-deficient mouse they averaged $20.24 \pm 1.31$ ms$^{-1}$ ($n = 11$). At 37°C, the velocities were $33.05 \pm 2.13$ ms$^{-1}$ ($n = 6$) in the wild-type mouse and $29.93 \pm 1.48$ ms$^{-1}$ ($n = 11$) in the APP-deficient mouse. In these experiments the APP-deficient mouse consistently had a slight slower conduction velocity, but the difference was not significant (Fig. 18b). Given that APP clustering only occurs in the CNS, the significant change of nerve conduction in the mutant spinal cord, but not sciatic nerve, implies that the clustered APP is an important factor that plays a specific role in modulation of the CNS action potentials.
According to the present invention, by influencing the expression of APP and its fragments and derivatives, and that of molecules, cellular components, drugs and/or antibodies that interact with APP, its fragments and derivatives, many biological and medical events, functions and conditions, particularly neurological conditions, may be treated.

The expression of Nav1.6 sodium channel α subunit is abnormally downregulated in the CNS of APP null mice.

In the adult, Nav1.6 is the predominant α subunit of sodium channels that locate at CNS NORs (Salzer, 2003). As the conduction velocities of compound action potentials is abberantly reduced in the APF+ spinal cord, the present inventors ascertained as to whether there might be differences in the level of Nav1.6 expression between APF+ mice and their wt littermates. Western blot results showed that the expression of Nav1.6 was abnormally decreased in the brain in APF− mice versus APP+/+ and APP−/− littermates (Fig. 24). Together, the abnormal changes in locomotor activity, forelimb grip strength (Zheng et al., 1995), nerve conduction, and Nav1.6 expression in the APF+ mice (Fig. 18 and Fig. 24) strongly imply that APP may act as a specific modulator of Nav1.6 at the CNS NORs.

Example 9 - VTPEER domain of APP increases sodium current expression in Xenopus oocytes expressing the Nav1.6 sodium channel α subunit.

The present inventors also determined which domain(s) in APP could involve in increment of sodium current expression in Xenopus oocytes expressing the Nav1.6 sodium channel α subunit. APP intracellular domain (AICD; X. Cao and Südhof (2001), Go protein-binding domain (GB; H657-K676 of APP-695; Nishimoto et al., 1993; Brouillet et al., 1999), Fe65 binding domain (Fe65BD; v 666γ 686; Ermekova et al. Q97), and C31 domain (C31 : A 664-H 695, Liu et al.,
2000; Figure 25) were constructed and coexpressed with the Nav1.6 a subunit in *Xenopus laevis* oocytes, respectively.

Interestingly, all the intracellular domains, including AICD, GB, Fe65BD, and C31, increased the peak sodium current (Fig. 26 a to d), but control injection of cRNAs for their reverse sequences did not.

Given that VTPEER is a common overlapped peptide sequence in the AICD, GB, Fe65BD, and C31, the inventors further coexpressed this peptide with the Nav1.6 a subunit in *Xenopus laevis* oocytes. Notably, similar to full length APP and other intracellular domains, VTPEER increased the peak sodium current as well (Fig. 26e; Figure 25). All the intracellular domains had no significant effect on activation, inactivation or recovery from inactivation (Figure 28). To further confirm the role of the peptide in modulating sodium currents, the inventors chemically synthesised VTPEER peptide and measured its effects in a human embryonic kidney 293-cell line stably expressing human Nav1.6 (Oliveira et al., 2004). Consistently, the expression level of sodium current was also significantly increased in the Nav1.6-transfected 293-cells after treatment with the synthesised peptide in comparison with before peptide treatment (Fig. 26f). These data demonstrate that VTPEER peptide mediates the effects of APP on sodium currents.

Example 10 - Go protein is involved in the modulation of sodium currents by APP.

Various sodium channels are modulated by G proteins (Komwatana et al., 1996; Ma et al., 1997; Lu et al., 1999). The APP intracellular portion contains a Go protein-binding (GB) domain, His\(^{657}\)-Lys\(^{676}\) of APP-695 (Nishimoto et al., 1993; Lang et al., 1995; Okamoto et al., 1995; Brouillet et al., 1999).
Further to their finding that APP GB domain increases the sodium current expression, the inventors postulated that Go protein might mediate the APP-dependent modulation of sodium currents. The inventors tested this by coexpressing the Nav1.6 α subunit and a G203T dominant-negative mutant of the human G-protein αoB subunit (GoDN) together with: (i) APP (Fig. 27a); (ii) AlCD (Fig. 27b); (iii) GB (Fig. 27c), (iv) Fe65BD (Fig. 27d), (v) C31 (Fig. 27e), and (vi) VTPEER (Fig. 27f), respectively, in Xenopus laevis oocytes. Co-expression with GoDN reduced the sodium current in the presence of APP and all its intracellular domains as well as the VTPEER peptide (Fig. 27). Control injection of GoDN and the Nav1.6 α subunit without APP did not change the sodium current. Accordingly, these data demonstrate that the effects of APP on sodium currents involve Go proteins.

Discussion for Examples 1-10

The present inventors have found that APP is clustered specifically at the NORs in the CNS, but not PNS. Consistently, conduction velocities are significantly impaired only in the CNS, but not PNS, in APP mutant mice. Moreover, APP positively modulates the function of sodium channels, increasing sodium currents through its intracellular VTPEER peptide via a Go protein-coupled pathway.

APP is a novel component at the CNS NORs.

During myelination, various axonal and glial molecules redistribute themselves to take up defined domains through dynamic axoglial interaction (Salzer 2003). Over the past decades, the molecular composition corresponding to the morphological domains of the myelinated axon has been extensively studied, with a major focus on the cell adhesion molecules. For instance, F3/contactin, a
member of the immunoglobulin superfamily, has been implicated in molecular
communication in both nodal and paranodal sites of the CNS, via interacting
with sodium channels and Caspr/paranodin, respectively. TAG-1/TAX, from the
same L1 cell adhesion molecule subfamily as F3/contactin, has been suggested
to be a constituent of juxtaparanodal formation (Traka et al., 2002 and Poliak et
al., 2003). The sodium channel β subunits, which associate with α subunits of
voltage-gated sodium channels at the NORs, have an extracellular
immunoglobulin-like loop and act as cell adhesion molecules (McEwen and
Isom, 2004). APP is expressed predominantly in axons and is upregulated on
neural injury and has been utilized as a marker for axonal degeneration. The
present work shows that APP is aggregated specifically at the NORs in the
CNS, but not PNS. The clustering of APP occurs later than the clustering of
sodium and potassium channels. This aggregation requires the integrity of
distinct axonal domains as evidenced in both physiological and pathological
conditions. Conversely, APP is not required for clustering of other nodal
molecules such as tenascin-R, Caspr and sodium channels, as clustering of
these molecules still occurs in APP null mice. APP clustering starts from P10
during myelination and disappears after demyelination in two animal models.
Thus, nodal APP clustering in the CNS may be the most important factor related
to the phenotype of reduced conduction velocities in the CNS, but not PNS, in
APP mutant mice. However, the molecular mechanisms underlying the
clustering of APP at CNS NORs remain to be explored.

Potential role of APP in modulating sodium channels

The structure of the NORs is formed by the halt of lateral expansions of two
apposing oligodendrocytes in the CNS. The voltage-gated sodium channels are
highly concentrated at this region with a density of 1,500 molecules/μm², but of
less than 100 molecules/μm² in the non-nodal areas (Rosenbluth 1999). Since
the myelin sheath insulates the wrapped portion of the axon, the action potential
is conducted in a saltatory manner, thus significantly increasing the conduction
speed. Sodium channels are responsible for depolarizing and repolarizing events during transmission of the action potential along axons. The fine modulation of sodium channels at the NORs is essential for precise saltatory conduction along myelinated axons. At nodal regions, sodium currents are positively modulated by axonal molecules, such as F3/contactin, β subunits of sodium channels, and glia-derived molecules, such as tenasin-R and -C (Kazarinova-Noyes et al., 2001; Isom et al., 1995; Srinivasan et al., 1998; and Ma et al., 2007). APP does not act as a β subunit of sodium channels, as the increase in the sodium current in the presence of APP was not in a dose dependent manner (Figure 29). The increase in the sodium current did not require the β subunits of the sodium channel but was augmented by the β subunits. In the presence of the βi subunit, APP also induced a shift to the right in the activation of the channel while, in the presence of the β2 subunit, APP accelerated current recovery from inactivation (Figures 14-16). Tenasin-R and its neuronal receptor, F3/contactin, which are extracellular molecules, interact with sodium channels at the NORs (Kazarinova-Noyes et al., 2001). As positive modulators of sodium channels, tenasin-R acts in a β subunit-dependent manner and F3/contactin increases membrane insertion of the α subunit. Similar to tenasin-R and F3/contactin, APP clusters at CNS NORs and increases Nav1.6 sodium currents in oocytes. The APP-induced increase in the sodium currents is mediated by its intracellular VTPEER peptide, a common domain shared by AICD, GB, Fe65BD, and C31. Consistent with the augmentation of sodium currents by APP, spinal conduction velocities are impaired in APP null mice.

The APP-induced increase in the sodium currents is also mediated by the His657-Lys676 Go protein binding domain in the AICD of APP-695 and appears to involve the heterotrimeric G protein Go. This G protein binding domain is known to selectively activate the heterotrimeric G protein Go with which it complexes (Nishimoto et al., 1993; Brouillet et al., 1999). Although APP modulation of
Nav1.6 via G protein has not previously been reported, our findings are consistent with G protein-mediated modulation of various other sodium channels (Komwatana et al., 1996; Ma et al., 1997; Lu et al., 1999).

The nodal Aβ hypothesis.

Models of amyloid deposition in Alzheimer’s disease have envisaged exclusively synaptic accumulation. Notably, specific APP clustering at the CNS NORs implicates a potential role for the NORs in the pathogenesis of AD. The exact mechanisms by which APP contributes to the development of dementia are unknown but it is thought that cleavage of APP by secretases leading to formation of a smaller peptide, β amyloid (Aβ), is a crucial step. Deposition of Aβ, is considered to be a marker of the pathology of AD and is associated with other pathological markers of AD such as cell death in the CNS, accumulation of amyloid plaques, and the appearance of neurofibrillary tangles. APP is enriched in presynaptic terminals (Ferreira et al 1993). Although the release of component peptides of APP, including Aβ deposition, has been thought to occur predominantly at synapses (Ferreira et al (1993), increasing evidence strongly suggests that an important component of AD is damage to myelin. β1 and β2 subunits of sodium channels, two nodal molecules, have been recently reported to be substrates of β site amyloid precursor protein-cleaving enzyme (BACE1) and γ-secretase (Kim et al., 2005 and Wong et al., 2005). It has recently been reported that BACE1 controls myelination in the PNS (Willem et al. (2006). In AD patients, the entorhinal cortex, from where the perforant pathway originates, is one of the most severely affected areas, particularly at earlier stages of the disease. Moreover, electrical activity within the pathway modulates interstitial fluid Aβ levels, which can be blocked by tetrodotoxin (TTX), a specific sodium channel blocker (Cirrito et al., 2005). NORs are the naked regions of the axolemma and intermittently distribute along myelinated axons, implying potential interactions between exposed axonal molecules and the extracellular partners. According to the observation that APP is specifically located at the CNS NORs, we propose a nodal Aβ hypothesis for AD: that the NORs are important Aβ releasing sites, which correlate with its deposition along the perforant pathway at the onset of AD.
Example 11

TAG-1 acts as a functional ligand for APP to trigger AICD transcriptional activation and negatively modulate neurogenesis. The present inventors have identified TAG-1 (contactin-2), a member of the F3/contactin family of glycolphosphatidylinositol-linked immunoglobulin superfamily adhesion molecules, as a functional ligand of APP. Cell adhesion assays and biochemical approaches established that TAG-1 and APP interact to trigger AICD's transcriptional activity in a γ-secretase-dependent manner. Further, both APP and TAG-1 were shown to co-localize in the neural stem cell niche of the fetal ventricular zone and in addition, neurogenesis is enhanced in neural precursor cells isolated from TAG-1-null, APP-null and TAG-1/APP double knock-out mice.

Cell adhesion assay

The following cell adhesion assays suggest a potential role between APP and TAG-1 in contrast to F3. When F3-transfected CHO (Fig. 30Ad and Fig. 30B) or control non-transfected CHO cells (Fig. 30 Ae and Fig. 28B) were seeded onto APP-Fc (recombinant APP extracellular domain in fusion with the Fc part of the Fc part of immunoglobulin) spotted culture dishes, little adhesion was observed. Interestingly, when TAG-1, or TAX (the human homolog of TAG-1), transfected CHO cells were seeded onto the culture dish dotted with APP-Fc, cells readily adhered to the APP protein spot (Fig. 30Aa and Fig. 30B), indicating that APP binds to TAG-1 or TAX. This adhesion could be blocked by pre-treating the cells with anti-TAG-1 antibody (Fig. 30Ab, Fig. 30B) or the culture dish with anti-APP antibody (22C1 1; Fig. 30Ac, Fig. 30B), indicating that the interaction of TAG-1 with APP contributes to the adhesion.
The reciprocal adhesion assays were performed by plating APP-transfected CHO cells onto culture dishes with TAG-1-Fc protein spots. Similar adhesion of the cells to the protein spot were observed (Fig. 30Af and Fig. 30B), and the adhesion was blocked by neutralization of coated TAG-1 protein spots (Fig. 30Ag and Fig. 30B) or cell membrane-bound APP (Fig. 30Ah and Fig. 30B) with their respective antibodies. Similarly, control non-transfected CHO cells did not adhere to the TAG-1 protein spot (Fig. 30Ai and Fig. 30B).

To identify the APP binding domains in TAX, GST fusion proteins of the 6 immunoglobulin Ig domains (TAXIg-GST) and 4 fibronectin type III (FNIII) repeats (TAXFNIII-GST) of TAX were used as coated protein substrates for APP-transfected CHO cells. The cells bound to spots of both proteins (Fig. 28Aj and i), indicating that APP has at least two binding sites located in the TAG-1 Ig domains and FNIII repeats. Consistently, control non-transfected CHO cells adhered to neither these two proteins (Fig. 30Ak and m) nor GST (Fig. 30An).

**GST or Fc pull down assay**

Immunoprecipitation and Fc or GST pull-down assays were performed in adult mouse brains to further confirm the interaction between TAG-1 and APP. The results showed that TAG-1 antibodies could precipitate APP from the mouse brain and vice versa (Fig. 31a). Consistent with the cell adhesion results, APP-Fc precipitated TAG-1 from the mouse brain (Fig. 31a) and both TAXIg-GST and TAXFNIII-GST precipitated APP from the mouse brain as well as APP transfected CHO cells (Fig. 31b). Together, these results demonstrate that APP and TAG-1 bind to each other.

**TAG-1 and AICD's transcriptional activity**

The activation of AICD release and transcriptional activity is thought to be triggered by external cues. However, ligands for the extracellular portion of APP
that affects its proteolytic processing and signalling cascade have not been functionally characterised.

The inventors studied the effect of TAG-1 on AICD's transcriptional activity. A previously reported luciferase reporter system where Gal4 is fused to the C-terminal of APP (Cao and Südhof 2001) was introduced into CHO and L1-, or TAG-1- or TAX-transfected CHO cells. A significant upregulation of the reporter activity was observed in both TAG-1- and TAX-transfected CHO cells, but not in the control CHO cells as well as L1-transfected CHO cells (Fig. 32a). Notably, a specific γ-secretase inhibitor reduced the TAG-1 triggered AICD transcriptional activity in a dose-dependent manner (Fig. 32b). That AICD luciferase activity is significantly increased by transfection with APP in both TAG and TAX transfected CHO cells suggest that the interaction between APP and TAG-1 is a critical step in triggering AICD activated transcription.

Co-localisation of APP and TAG-1

The expression of TAG-1 and APP in E14 brain was studied by immunofluorescence (IF) labelling using antibodies against TAG-1 (a polyclonal anti-TAG-1) and APP (C7). In the E14 mouse brain, IF showed that APP (Fig. 33b, b', c and c') or TAG-1 (Fig. 33e, e', f and f) were co-localized with nestin (Fig. 33a and d), a neural progenitor marker, in the walls of the lateral ventricles (LV). Moreover, double IF showed that APP (Fig. 33h and h') and TAG-1 (Fig. 33g and g') were co-expressed (Fig. 33i and i') in the LV walls. These results demonstrate that both TAG-1 and APP are expressed by NPCs in the neural stem cell niche.

In addition, NPCs from E14 mouse telencephalic ventricular walls were isolated. These cells were double-stained for APP (Fig. 34a' and c') or TAG-1 (Fig. 34b' and d') and the neural progenitor markers nestin (Fig. 34a and b) or Sox2 (Fig.
The results suggest that both APP and TAG-1 are expressed by the NPCs.

Further, the cells were double-stained for APP (Fig. 34e) and TAG-1 (Fig. 34e'), and the results indicate that APP and TAG-1 are co-localized in NPCs. Consistent with these observations, Western blot analysis showed that TAG-1 and APP bands were detected in total mouse brain and NPCs (Fig. 34f). Together, these results demonstrate that APP and TAG-1 are coexpressed in fetal NPCs.

**TAG-1 /APP signaling pathway in modulation of neurogenesis.**

NPCs were isolated from the E14 telencephalic ventricular walls of APP-/-(Fig. 35A) and TAG-1-/-(Fig. 35B) mice, respectively. After 7 to 8 days *in vitro* differentiation, cells were double-stained for TUJ1 (green; Fig. 35Aa and b; 35Ba and b) or MAP2, two markers for mature neurons (not shown), and DAPI (blue; Fig. 35Aa and b; Ca and b). Both TUJ1- and MAP2-positive cells are significantly increased in APP (about 40%; Fig. 35Bc and d) and TAG-1 (about 45%; Fig. 35Bc and d) deficient mice compared with wild-type littermates. These results demonstrate that the TAG-1/APP signaling pathway negatively modulates neurogenesis.

The inventors generated TAG-1-/APP-/- mice to confirm that TAG-1/APP signaling plays a negative role in modulating neurogenesis. Using the TAG-1-/APP-/- fetal NPCs, the inventors studied whether AICD transcriptional activity could be also modulated by the TAG-1/APP interaction. After transfection with the APP luciferase reporter system, TAG-1-/APP-/- NPCs were cultured as monolayers loaded onto culture dishes co-coated with TAG-1-Fc, F3-Fc, or L1-Fc and laminin. Interestingly, TAG-1-Fc, but not F3-Fc, LI-Fc, and laminin, strongly triggered AICD reporter activity (Fig. 36Aa). A specific γ-secretase
inhibitor blocked the induction of AICD transcriptional activity by TAG-1 in a dose-dependent manner (Fig. 36Ab), indicating that the \( \gamma \)-secretase is involved in the TAG-1 triggered RIP process in the TAG-1-/-/APP-/- fetal NPCs.

Similar to the observations in both TAG-1- and TAX-transfected CHO cells, these experiments further demonstrate that AICD transcriptional activity is also regulated by the interaction of TAG-1 and APP in NPCs. Notably, consistent with our observations in singly deficient mice, TUJ1- positive cells were significantly increased in TAG-1-/-/APP-/- (about 40 %; Fig. 34Bb and c) mice compared with wild-type littermates (Fig. 36Ba and c). Thus, given that neurogenesis is significantly promoted in NPC cultures isolated from TAG-1 and APP deficient mice as well as TAG-1/APP doubly deficient mice, the results strongly support the view that the TAG-1/APP signaling pathway negatively regulates neurogenesis during early CNS development.

The molecular structure and dimerization of APP suggest that APP is a cell surface receptor that can be activated by ligand binding (Kang et al 2004). In the present study, the inventors have shown that TAG-1, a member of F3/contactin family, is a functional ligand of APP. Similar to F3/contactin triggered NICD transcriptional activity, this binding promotes AICD transcriptional activity in a \( \gamma \)-secretase-dependent manner (Fig. 37). Moreover, the inventors have demonstrated that the TAG-1/APP signaling pathway is involved in negatively modulating neurogenesis (Fig. 37). These findings are important in the context of AD because abnormal processing of APP could also lead to aberrant AICD generation, which may be linked to abnormal gene expression.
Experimental Procedures

Antibodies

Antibodies used in this study were: rabbit TAG-123; mouse TAG-1 (4D7; Dr. A. Furley); goat TAG-1 (R&D system; Santa cruz); mouse APP (22C11; Chemicon); rabbit APP (C8; Dr. DJ Selkoe); rabbit APP C-terminal (751-770; C20), goat APP (Calbiochem); nestin, sox2, MAP2 (Chemicon); GFAP (Dako); β-tubulinIII (Tuj1; Sigma).

Production of Fc and GST fusion proteins

Mouse APP 695 cDNA encoding for the neuronal isoform of APP (Dr S. Sisodia, University of Chicago, USA) was subcloned into the pblue Bac vector using the BamHI and Sacl restriction sites. To generate the fusion protein containing the extracellular domain of APP with the Fc part of human immunoglobulin G at its COOH-terminal end (APP-Fc), primers for the Sacl restriction site at the 5’end (CTGACGGAACCAAGACCACCG; SEQ ID NO: 23) and for the COOH terminal end of the APP extracellular domain (terminating at amino acid position 624; SWISS-Prot accession number P12023) at the 3’ end (GCTGAAGATGTTGGTTCGAACAAA; SEQ ID NO: 24) were used, introducing a new BcII restriction site at the 3’end. This vector was used to stably transfect CHO K1 cells according to published procedures (Chen et al 1999).

A soluble form of TAG-1-Fc recombinant protein was produced in 293T cells. The signal sequence of the GPI-anchor of mouse TAG-1 was substituted with human IgG-Fc followed by a termination codon. The recombinant cDNA was inserted at the Hind III-Not I sites of pDX, a modified pcDNA3 vector with an amplification-promoting sequence (APS) upstream of the CMV site (Hemann et al 1994). The vector was introduced into 293T cells.
Fc proteins were purified via the Fc-tag using Protein-A column chromatography as described (Chen et al 1999). The sequences of Ig and FNIII domains of TAG-1 were inserted into the pGEXKG vector to produce the GST-tagged fusion proteins. The recombinant vectors were introduced into the TOP10 strain of *E. coli*, which was subsequently induced by IPTG (Bio-Rad). The recombinant GST fusion proteins were purified by using GST beads (Sigma) as instructed by the manufacturer.

Cell adhesion assays

CHO cells were stably transfected with pcDNA 3.1 (-) containing the human cDNA sequence of APP 695, the predominantly neuronal expressed isoform of APP. APP, F3, TAX, TAG-1 and mock transfected CHO cells were cultured in DMEM containing 10% fetal calf serum. 35mm tissue culture petri dishes (Becton Dickinson) were coated with methanol solubilized nitrocellulose and then with proteins (12µM) for 2 hours at 37°C in a humidified atmosphere. Subsequently, the dishes were washed and blocked overnight with 2% heat-inactivated fatty acid-free BSA (Sigma). After rinsing the dishes, cells (TAG-1-transfected CHO or APP-transfected CHO) were plated in 2 ml of DMEM (Gibco) containing 10% fetal calf serum at a density of 1.5 x 10^6 cells/ml. At 0.5 hour (in the adhesion test), the cells were gently washed and fixed with 2.5% glutaraldehyde and stained with 0.5% toluidine blue (Sigma) in 2.5% sodium carbonate. Blockage of adhesion was carried out using polyclonal anti-TAG-1 (1:100) or anti-APP (1:100) antibodies for 0.5 h pre-incubation. Cells adhering to the various spots were photographed and counted. The results were analyzed by Newman-Keuls test with p<0.05 being considered significant.
GST or Fc pull-down assays

Freshly prepared cerebral hemispheres of adult rats were harvested and solubilized in 2% Triton X-100. The buffer homogenate was centrifuged at 13,000g for 1 hour at 4 °C and the supernatant was incubated for 45 min at room temperature with the glutathioneagarose or protein A-coupled agarose beads that had been incubated with TAG-1-Fc or APP-Fc. After washing the beads, proteins were eluted with SDS-PAGE sample buffer and immunoblotted with anti-APP or anti-TAG-1 antibodies, respectively.

Co-immunoprecipitation and Western blot analysis

Mice brains were lysed with RIPA buffer containing protease inhibitor cocktail (Roche). For immunoprecipitation, lysates were precleared with protein A-coupled agarose beads for 1 hrs and incubated with APP or TAG-1 antibodies together with protein A-coupled agarose for overnight at 4 °C. Samples were washed with washing buffer before the beads were re-suspend in SDS buffer and boiled for 3-5 mins. Samples were subjected to SDSPAGE. Western blot analysis was performed and developed with ECL reagents (Amersham).

Luciferase assays

The APP-Gal4 assay system has been previously described15. L1-, TAG-1-, TAXtransfected CHO cells as well as CHO cells were cotransfected with the following plasmids (i) pG5E1B-luc (Gal4 reporter plasmid, 0.1 µg DNA); (ii) pCMV-LacZ (β-galactosidase control plasmid, 0.05 µg DNA); (iii) pMstAPP (Gal4) (0.1 µg DNA); (iv) pCMV5-Fe65 (Fe65) (0.1 µg DNA), in 24-well dishes using an Effectene Transfection kit (Qiagen). Additionally, cells cultured in 24-well dishes were used for AICD-Gal4 luciferase reporter assays. For the transactivation assay in NPCs, wells were coated with LI-Fc, TAGI-Fc, F3-Fc or laminin protein (8nM), respectively. Each well received 10 times the amount
of DNA as used for the CHO cells and the transfection was performed using a Nucleofector System (Amaxa). To examine the function of γ-secretase in this transactivation, two different concentrations (2 or 4μM) of a γ-secretase inhibitor (L-685, 458; Calbiochem) were applied to transfected cells, while DMSO was used as a control. The β-galactosidase expression plasmid pCMV/β-Gal was included to monitor the transfection efficiency. Cells were lysed at 24 hours after transfection and assayed using the Steady-Glo Luciferase Assay Kit (Promega).

**APP- and TAG-1-deficient mice**

APP-/- mice 26 and TAG-T-/- mice27 have been described previously. APP/TAG-1 doubly deficient mice were generated by intercrossing APP homozygous and TAG-1 homozygous (APP-/- X TAG-1-/-). Animals heterozygous for both loci were intercrossed to each other (TAG-1-/-7APP+/- X TAG-1-/-7APP+/-) to generate APP and TAG-1 doubly deficient (TAG-1-/-APP-/-) offspring.

**Culture of neural progenitor cells**

Telencephalic lateral ventricle walls isolated from E14 embryos were dissociated and neurosphere forming cells were cultured in DMEM/F12 (Gibco) containing N2 supplement, 20ng/ml bFGF and 20ng/ml EGF. For differentiation assay, second passage neurospheres were collected and dissociated into single cells. The cell suspension was seeded into 24-well dishes with 20,000 cells per well and was induced in DMEM/F12 culture medium containing N2 and 0.5% fetal calf serum for 7-8 days.
Immunocytochemistry and quantitation

Immunocytochemistry on cultured cells and immunostaining on tissue sections were performed as previously described. For quantitation of immunofluorescence, images of fields of cultured cells were captured by digital photomicrograph under a 1.6x1.0X objective systematically from top-to-bottom and left-to-right across the entirety of each coverslip.

All labeled cells were then counted in each photomicrograph. The proportion of neurons was quantified as the numbers of Tuj1+ or MAP2+ cells divided by the total number of DAPI+ cells in the same fields. Each experiment was repeated on 3 to 7 mice.

The statistics were performed using one-way-ANOVA or Student's t test, as appropriate. In all the graphs, the error bars indicate standard error of the mean (SEM). *: P<0.05;**: P<0.001.

Although the present invention has been described in detail with reference to examples above, it is understood, that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is only limited by the following claims. All cited patents, patent applications and publications referred to in this application are herein incorporated by reference in their entirety.
References


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Claims

1. A method of treating at least one medical condition in a subject, the method comprising modulating the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment.

2. The method according to claim 1, wherein the modulating is by decreasing the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment.

3. The method according to claim 1 or 2, wherein the modulation of APP, its derivative or a fragment thereof is by modulating at least one molecule, protein, and/or peptide that interacts with amyloid precursor protein (APP), APP derivative or APP fragment.

4. The method according to any one of the preceding claims, wherein the modulation of amyloid precursor protein (APP), APP derivative or APP fragment comprises modulating at least one cellular component that interacts with amyloid precursor protein (APP), APP derivative or APP fragment.

5. The method according to claim, wherein the at least one cellular component that interacts with amyloid precursor protein (APP), APP derivative or APP fragment is selected from TAG-1, sodium channels, and sodium channel complex protein.

6. The method according to any one of the preceding claims, wherein the modulation of amyloid precursor protein (APP), APP derivative or APP fragment comprises modulating at least one cellular component selected from the group consisting of TAG-1, Caspr2, NgR, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ, NG2, sodium channel and Go protein(s).
7. The method according to any one of the preceding claims, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one drug, protein, peptide, antibody or molecule that interacts with amyloid precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium channels, or sodium channel complex protein(s).

8. The method according to any one of the preceding claims, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one drug, protein, peptide, antibody or molecule that interacts with amyloid precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium channels, TAG-1 complex protein(s), sodium channels complex protein(s), Caspr2, NgR, potassium channels, TN-R, TN-C, MAG, Nogo-A, phosphacan, F3, OMgp, RPTPβ and NG2.

9. The method according to any one of the preceding claims, wherein the modulating comprises administering at least one pharmaceutically and/or therapeutically effective amount of amyloid precursor protein (APP), APP derivative or APP fragment.

10. The method according to claim 9, wherein the APP derivative or APP fragment is Aβ, soluble APP (sAPP), APP-695, AICD or VTPEER peptide.

11. The method according to any one of the preceding claims 1 to 8, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one compound selected from the group consisting of TAG-1, anesthetic(s), toxin(s) and Go protein(s).
12. The method according to any one of claims 1 to 8, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one drug or molecule selected from local anaesthetic(s), veratridine, batrachotoxin, scorpion toxins, sea anemone toxin, conus toxins, saxitoxin, tetrodotoxin, antiepileptic drug(s), antiarrhythmic drug(s), antibodies to sodium channel proteins, antibodies to Caspr, and peptide fragments of APP.

13. The method according to any one of the preceding claims, wherein the modulation of amyloid precursor protein (APP), APP derivative or APP fragment is to prevent and/or reduce beta amyloid (Aβ) deposition, improve and/or facilitate nervous tissue repair, improve and/or facilitate myelination, to modulate stem cell function(s) and/or to modulate sodium channels function(s).

14. The method according to any one of the preceding claims, wherein the at least one medical condition is selected from the group consisting of Alzheimer's disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders and demyelination disorders.

15. The method according to any one of the preceding claims, wherein the modulating results in modulation of axonal conduction of action potential(s) for the treatment of pain and/or epilepsy.

16. The method according to any one of the preceding claims, wherein the modulating results in the induction of analgesia and/or local anaesthesia.
17. A method of detecting and/or quantitating the presence of, predisposition to, and/or severity of, a neurological condition in a subject, the method comprising:
   (a) providing at least one sample from a subject;
   (b) determining the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment;
   (c) comparing the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment, with that of at least one control, a difference in expression and/or activity indicating the presence of, or predisposition to, and/or severity of a neurological condition in the subject.

18. A method of monitoring the efficacy of a treatment for a neurological condition in a subject, the method comprising:
   (a) providing at least two samples from a subject, each sample obtained at different time points;
   (b) determining the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment; and
   (c) comparing the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment, in the at least two samples.

19. A method of prognosticating the outcome of a neurological condition in a subject, the method comprising:
   (a) providing at least one sample from a subject;
   (b) determining the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment;
   (c) comparing the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment, with that of at least one control, a difference in expression and/or activity indicating the prognosis of a neurological condition in the subject.
20. A method of inducing analgesia and/or anesthesia in a subject, the method comprising modulating the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment.

21. The method according to claim 20, wherein the modulating comprises decreasing the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment.

22. An in vitro or in vivo method of modulating proliferation, differentiation and/or activity of at least one stem cell, the method comprising modulating the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment, in the at least one stem cell.

23. The method according to claim 22, wherein the modulating comprises decreasing the expression and/or activity of amyloid precursor protein (APP), APP derivative or APP fragment, in at least one stem cell.

24. The method according to claim 20 to 23, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one compound selected from the group consisting of TAG-1, anesthetics, toxins and Go protein(s).

25. The method according to any one of claims 20-24, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one drug or molecule selected from local anaesthetic(s), veratridine, batrachotoxin, scorpion toxins, sea anemone toxin, conus toxins, saxitoxin, tetrodotoxin, antiepileptic drug(s), antiarrhythmic drug(s), antibodies to sodium channel proteins, antibodies to Caspr, and peptide fragments of APP.
26. The method according to any one of claims 20 to 25, wherein the method is for neural system repair in a subject.

27. A method of detecting and/or quantitating the presence of, predisposition to, and/or severity of, at least one condition related to beta amyloid (Aβ) deposition and/or sodium channel dysfunction in a subject, the method comprising:
   (a) measuring the nerve and/or axon conduction velocity in the subject;
   (b) comparing the nerve and/or axon conduction velocity with that of at least one control, a difference in velocity indicating the presence of, or predisposition to, and/or severity of at least one condition related to Aβ deposition and/or sodium channel dysfunction in the subject.

28. The method according to claim 27, wherein the method is for detecting and/or quantitating the presence of, predisposition to, and/or severity of Alzheimer's disease (AD).

29. An in vitro or in vivo method of modulating the activity of amyloid precursor protein (APP), APP derivative or APP fragment, comprising administering at least one drug, protein, peptide, antibody or molecule that interacts with amyloid precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium channels, sodium channel complex protein(s).

30. The method according to claim 29, wherein the modulating comprises administering a pharmaceutically and/or therapeutically effective amount of at least one compound selected from the group consisting of TAG-1, anesthetic(s), toxin(s) and Go protein(s).
31. The method according to claim 29 or 30, wherein the method is for treating at least one condition selected from the group consisting of Alzheimer's disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders and demyelination disorders.

32. An in vitro or in vivo method of modulating sodium channel(s) function(s) comprising administering at least one drug, protein, peptide, antibody or molecule that interacts with amyloid precursor protein (APP), APP derivative or APP fragment, TAG-1, sodium channels, or sodium channel complex protein(s).

33. The method according to claim 32, wherein the method is for modulating APP expression and/or activity), and/or for the treatment of at least one neurological disorder.

34. The method according to claim 32 or 33, wherein the method is for the treatment of acute and/or chronic pain disorder(s), induction of analgesia, treatment of epilepsy and/or convulsion(s), treatment of disorder(s) of the rhythm of the heart, and/or induction of anaesthesia and/or analgesia.

35. A pharmaceutical composition for treating at least one APP and/or sodium channel related medical condition comprising at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein(s), Caspr, Caspr derivative, Caspr fragment, anesthetic(s), toxin(s) and Go protein(s); and optionally at least one pharmaceutically acceptable diluent, carrier and/or excipient.
36. A pharmaceutical composition according to claim 35 wherein the APP derivative or fragment is Aβ, soluble APP (sAPP), APP-695, AICD or VTPEER peptide.

37. The pharmaceutical composition according to claim 34 or 35, wherein the medical condition is at least one condition selected from the group consisting of Alzheimer's disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders and demyelination disorders.

38. Use of at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s), toxin(s) and Go protein(s), for the preparation of a medicament for the treatment of an APP and/or sodium channel related medical condition.

39. Use according to claim 38, wherein the APP derivative or APP fragment is Aβ, soluble APP (sAPP), APP-695, AICD or VTPEER peptide.

40. A compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s), toxin(s) and Go protein(s) for use in the treatment of an APP and/or sodium channel related medical condition.
41. A compound according to claim 40, wherein the APP fragment of derivative is Aβ, soluble APP (sAPP), APP-695, AICD or VTPEER peptide.

42. A compound, according to claim 40 or 41, for use in the treatment of Alzheimer's disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders or demyelination disorders.

43. A kit comprising at least one compound selected from the group consisting of: APP, APP derivative, APP fragment, antibody to APP, TAG-1, at least one TAG-1 complex protein, a TAG-1 homologous protein or fragment thereof, antibody to TAG-1, sodium channel(s), at least one sodium channel complex protein, Caspr, Caspr derivative, Caspr fragment, anesthetic(s) toxin(s) and Go protein(s), for detecting and/or diagnosing at least one APP and/or sodium channel related medical condition or for the treatment of at least one APP and/or sodium channel related medical condition.

44. A kit according to claim 43, wherein the kit comprises Aβ, soluble APP (sAPP), APP-695, AICD or VTPEER peptide.

45. A kit according to claim 43 or 44, for the treatment of Alzheimer's disease, multiple sclerosis, brain injury, spinal cord injury, axonal injury-related disorders, epilepsy, neurodegenerative disorders or demyelination disorders.
FIGURE 1

Oligodendroglial loop

Axon, Internode, Juxtaparanode, Paranode
FIGURE 2
FIGURE 3
FIGURE 5
FIGURE 7

[Bar chart showing APP/Kch (%) for P10, P15, P21, P2M.]

FIGURE 8

[Bar chart showing Percentage of APP positive node for APP and Kch.]
FIGURE 9

[Image of microscopic samples labeled WT and KO, with a graph showing a comparison of g ratio between WT and KO with a significant difference indicated by "**".]
FIGURE 11
FIGURE 13

(a) Diagram showing current and voltage relationships.

(b) Graph showing normalized current vs. voltage for different conditions.

(c) Graph showing normalized conductance vs. voltage for different conditions.

(d) Graph showing normalized current vs. prepulse voltage for different conditions.

(e) Graph showing fractional recovery for different conditions.
FIGURE 17

a

b

WT (n=10)
KO (n=19)

Normalized Conductance

Voltage (mV)

Voltage (mV)

c

d

WT (n=13)
KO (n=20)

Normalized Conductance

Prepulse Voltage (mV)

Fractional Recovery

Recovery Time (ms)
FIGURE 19

A

AFP

γ-actinin

100 Da

50

F3 F5 P7 P8 P10 P15 P21 F30 Adult

B

10 μm
FIGURE 21

![Graph showing the relationship between axon diameter and g ratio for APP KO and APP WT.](image)

FIGURE 22

(a) Axon Diameter (um) vs. WT, TG

(b) Fiber Diameter (um) vs. WT, TG
FIGURE 24

Nav1.6

γ-tubulin
FIGURE 25

Full-length APP695

AICD50

C31

G0BD

Fe65 BD

Val^{67}-Arg^{672}

VMLKKQYTSIHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

AAVTPEERHLSKMQQNGYENPTYKFFEQMQN

HHGVVEVDAAVTPEERHLSK

VTPEERHLSKMQQNGYENPTY

VTPEAR
FIGURE 28

a

Normalized Current

b

Voltage (mV)

Fractional Recovery

Recovery Time (ms)

Nav1.6 (n=39)
Nav1.6+ACD (n=39)

Nav1.6 (n=20)
Nav1.6+ACD (n=21)
**FIGURE 30**

B

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**Number of binding cells**

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**CHOTAG1** and **a-TAG1** have the highest binding to APP-Fc and TAG1-Fc, respectively.
FIGURE 32

a

Fold change (%)

400

300

200

100

0

CHO  CHOL1  CHOTAX  CHOTAG1

b

Fold change (%)

120

80

40

0

DMSO  2μM γ-inhibitor  4μM γ-inhibitor

**

**
FIGURE 35
**FIGURE 37**

APP → TAG-1 → ?-secretase → Fe65 → sAPP, AICD, Aβ → Tip60 → Transcriptional activation → Inhibition of neurogenesis
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 38/02 (2006.01)  A61P 25/00 (2006.01)  G01N 55/50 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. MINIMUM DOCUMENTATION SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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)


C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>5, 11, 16, 30, 34</td>
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</table>

Further documents are listed in the continuation of Box C

X See patent family annex

* "A" Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "P" 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
  "E" earlier application or patent but published on or after the international filing date
  "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
  "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)
  "X" 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
  "O" document referring to an oral disclosure, use, exhibition or other means
  "X" document member of the same patent family
  "P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search

10 October 2007

Date of mailing of the international search report

2 B OCT 2007

Name and mailing address of the ISA/AU

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Telephone No: (02) 6283 3174

Form PCT/ISA/210 (second sheet) (April 2007)
## INTERNATIONAL SEARCH REPORT

### DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>EP 564946 A1 (MILES INC.) 13 October 1993&lt;br&gt;Whole document, especially abstract, page 3 lines 16-21, page 4 lines 2-page 5 line 9, Figure 1, Example 5</td>
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<td>WO 2005/102440 A2 (INNOSPINE, INC.) 20 October 2005&lt;br&gt;Abstract, claim 26</td>
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<td>RASBAND, Matthew N. et al., Dysregulation of axonal sodium channel isoforms after adult-onset chronic demyelination. Journal of Neuroscience Research 2003, Vol. 73, No. 4, pages 465-470&lt;br&gt;Abstract</td>
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Form PCT/ISA/210 (continuation of second sheet) (April 2007)
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:

i.  [ ] Claims Nos.: 1-2
because they relate to subject matter not required to be searched by this Authority, namely:

2.  [X] Claims Nos.: 1-45 (partially)
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:
The claims relate to an extremely large number of possible compounds. The search has been carried out for those parts of the claims which appear to be supported and disclosed, namely the compounds listed in claims 5, 9-12, 30, 35-41, 43 and 44. Terms listed in claims 6 and 8 were also included, but the search will not cover all compounds that inherently modulate APP or these or any other cellular components that interact with it.

3.  [ ] Claims Nos.: 46-50
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:

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 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.  [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos:

Remark on Protest

☐ The additional search fees were accompanied by the applicant’s protest 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 2007)
This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX