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(54) **Title:** NICOTINIC CHANNEL SUBUNITS OF POLLINATOR INSECTS AND THEIR USES THEREOF

(57) **Abstract:** The present invention relates to the cloning of the nicotinic acetylcholine receptors (nAChR) subunits in species from the phylum arthropoda and in particular a pollinator insect such as *Apis mellifera* and their uses thereof.



NICOTINIC CHANNEL SUBUNITS OF POLLINATOR INSECTS AND THEIR USES THEREOF

FIELD OF INVENTION

- 5 The present invention relates to the expression of the nicotinic acetylcholine receptors (nAChR) subunits in species from the phylum arthropoda and in particular a pollinator insect such as *Apis mellifera* and their uses thereof.

BACKGROUND OF INVENTION

- 10 During the last decades, agriculture has changed to meet the increasing demand to produce food. There has been expansion of cultivated areas in monoculture and increases use of pesticides. The abusive use of pesticides is subjecting pollinator insects to stress as evidenced by a constant decrease in the density of these pollinator insects around agricultural fields in many parts of the world, thus causing economic losses.
- 15 Pollinators are crucial for the pollination of agricultural crops and natural areas around the world.

Among these pollinator insects, some insects issued from the order of *hymenoptera* or bees are considered excellent pollinating insects in agro-ecosystems because they visit many flowers on the same day. Among these pollinator insects, Bumble bees for
20 instance have already been red-listed and are in danger of extinction.

Another bee such as *Apis mellifera* or honey bee is interesting from an economic perspective because it provides products of great value, such as honey, propolis, royal jelly, wax, and apitoxin (bee venom).

The decline of the bee colonies and pollinator insects due to pesticides is not only
25 marked by the increase of their bee mortality but also modifications related to their memory or social behavior within the hives (Suchail S et al 2001 Environ Toxicol Chem 20 (11):2482-6; Gels JA 2002 J Econ Entomol; 95(4):722-8). A direct link

between the uses of phytosanitary products issued from the neonicotinoid family and the decline of the bee colonies has already been established. For this reason, France has decided the ban of these products from January the 1st 2016 while the United States won't deliver any more authorization of marketing any new derivate products from the neonicotinoid family. These products are known to target the nicotinic acetylcholine receptor (nAChR) channels.

Some toxicity assays have already been developed by oral or topical application of a substance on the insect however these assays are not reproducible and are not adapted to the test of a large diversity of compounds (OECD guidelines for the testing of chemicals OECD214 - September 1998). Consequently there is a need to develop *in vitro* high throughput assays highly reproducible to diagnose bee colonies health, to determine any toxicity within the hives or to screen new compounds not targeting bee colonies.

The present application indeed unravels sequences of nAChR subunits and the sequences of chaperone proteins from pollinator insects able to form a functional channel that can respond to a modulator. In addition, the present application discloses a method using the nucleic acid sequences of nAChR channel subunits and chaperone proteins from pollinator insects to analyze the effect of compounds on nAChR channel activity.

SUMMARY

One object of the invention is a nucleic acid sequence of at least one subunit α of the nicotinic acetylcholine receptor of an arthropod as set forth in SEQ ID NO: 1 to 9 or a variant thereof.

Another object of the invention is a nucleic acid sequence of at least one subunit β of the nicotinic acetylcholine receptor of an arthropod as set forth in SEQ ID NO: 10 to 11 or a variant thereof.

Another object of the invention is a nucleic acid sequence of the chaperone protein of the nicotinic acetylcholine receptor of an arthropod as set forth in SEQ ID NO: 12 to 15 or a variant thereof.

Another object of the invention is a vector comprising at least one nucleic acid sequence
5 as defined here above.

Another object of the invention is an isolated cell transfected with at least one vector as defined here above.

Another object of the invention is a functional nicotinic acetylcholine receptor (nAChR) channel comprising at least one subunit α of nAChR encoded by a nucleic acid
10 sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof as defined here above.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 1$ subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof.

15 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 3$ subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 4$ subunit encoded by the nucleic acid sequence as
20 set forth in SEQ ID NO: 4 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 5$ subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the
25 nicotinic acetylcholine receptor $\alpha 7$ subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

Another object of the invention is a functional nAChR channel comprising at least one subunit α encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof as defined here above and at least one chaperone protein encoded by the nucleic acid sequence selected from the group comprising SEQ ID NO: 12 to 15 or a variant thereof as defined here above.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 3$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the chaperone protein ric3 encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

10 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 3$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the chaperone proteins emc-6 and unc50 encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 and 14 or a variant thereof.

15 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 3$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the chaperone proteins emc-6, unc50, unc74 encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 to 15 or a variant thereof.

20 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 5$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 5 or a variant thereof and the chaperone protein ric3 encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

25 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 5$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 5 or a variant thereof and the chaperone proteins emc-6, unc50, unc74 encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 to 15 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 7$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 7 or a variant thereof and the chaperone protein ric3 encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

- 5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor $\alpha 7$ subunit encoded by the nucleic acid sequence consisting of SEQ ID NO: 7 or a variant thereof and the chaperone protein ric3 encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof and the chaperone proteins emc-6, unc50, unc74 encoded by the nucleic acid sequence
10 consisting of SEQ ID NO: 13 to 15 or a variant thereof.

Another object of the invention is a functional nicotinic acetylcholine receptor (nAChR) channel comprising at least one subunit α of nAChR encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof as defined here above and at least one subunit β encoded by a nucleic acid sequence
15 selected from the group comprising SEQ ID NO: 10 to 11 or a variant thereof as defined here above.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor $\beta 1$ subunit encoded by the
20 nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor $\beta 2$ subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 11 or a variant thereof.

- 25 In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor $\beta 1$ subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof and the

chaperone protein ric3 encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

Another object of the invention is a functional nAChR channel of an arthropod comprising at least one subunit α of nAChR encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof as defined here above, at least one subunit β of nAChR encoded by the nucleic acid sequence selected from the group comprising SEQ ID NO: 10 to 11 or a variant thereof as defined here above and at least one chaperone protein encoded by nucleic acid sequences selected from the group comprising SEQ ID NO: 12 to 15 or a variant thereof as defined here above.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

Another object of the invention is a vector comprising the channel as defined here above.

Another object of the invention is an isolated cell transfected with at least one vector as defined here above, and expressing the channel as defined here above, preferably said cell is an oocyte of *Xenopus*.

Another object of the invention is an *in vitro* method to determine the effect of a test compound on the modulation of activity of a nAChR channel of an arthropod comprising:

- a. contacting an isolated cell as defined here above with at least one test compound,
- b. measuring the effect of said test compound on the channel activity, and

comparing said effect to the effect without test compound or to the effect of a reference value, thereby determining a modulation of activity of said channel.

In one embodiment, said method is for determining the toxicity of a test compound on an arthropod.

5 Another object of the invention is an *in vitro* method for screening compounds that modulate the nAChR channel activity of an arthropod comprising:

a. contacting an isolated cell as defined here above with at least one test compound,

b. measuring the effect of said compound on the nAChR channel activity,

10 and

comparing said effect to the effect without test compound or to the effect of a reference value, thereby determining a modulation of activity of said nAChR channel.

Another object of the invention is a kit comprising at least one vector as defined here above, or an isolated cell as defined here above and reagents.

15

DEFINITIONS

In the present invention, the following terms have the following meanings:

- “**Agonists**” refer to compounds that bind to the receptor site where acetylcholine (ACh) or acetylcholine binding protein (AChBP) binds (is also referred as the “active” or “orthosteric” site) and activate it, resulting in increased channel conductance.

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- “**Antagonists**” refer to compounds that bind to the receptor site where ACh binds but do not activate it. Though they have no effect on their own, antagonists compete with ACh for binding and thereby inhibit its action, resulting in decreased channel activity.

25

- “**Positive allosteric modulators**” refer to compounds that bind to allosteric sites on the receptor complex causing increased efficiency of the main site and therefore an indirect increase in channel activity.

- “**Negative allosteric modulators**” refer to compounds that bind to allosteric site on the receptor complex causing decreased efficiency of the main site and therefore an indirect decrease in channel activity.
- “**Open channel blockers**” refer to compounds that block the ion channel pore once it is open, and thus prolong ligand-receptor occupancy, slow activation kinetics and inhibit ion flux in a subunit configuration-dependent and sensitization-state dependent manner.
- “**Non-competitive channel blockers**” refer to compounds that bind to or near the central pore of the receptor complex and directly block channel conductance through the ion channel.
- “**Am**” or “**Amel**” preceding any gene’s name refer to the pollinator insect *Apis mellifera*.
- “**About**” preceding a figure and/or a score means plus or less 10% of the value of said figure and/or score.
- “**Derivative**” or “**analog**” of a compound refers broadly to the modification or substitution of one or more chemical moieties on a parent compound and may include functional derivatives, positional isomers, tautomers, zwitterions, enantiomers, diastereomers, racemates, isosteres or stereochemical mixtures thereof.
- “**Identity**” when used in a relationship between the sequences of two or more nucleic acid sequences or amino acid sequences, refers to the degree of sequence relatedness between the sequences, as determined by the number of matches between strings of two or more base pairs of nucleic acid or amino acid residues. “Identity” measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., “algorithms”). Identity of related nucleic acid sequences or amino acid sequences can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.

- W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., SIAM J. Applied Math. 48, 1073 (1988). Preferred methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer programs. Preferred computer program methods for determining identity between two sequences include the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res. 12, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol. 215, 403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra). The well-known Smith Waterman algorithm may also be used to determine identity.
- **“Functional channel”, “functional expression”** refer to the synthesis and any necessary post-translational processing of at least one subunit of the channel and/or at least one of the chaperone protein in an isolated cell so that the subunit or its chaperone protein is inserted properly in the cell membrane and is capable of conducting ions in response to an exposure to appropriate pharmacological agents or test compounds.
 - **“Minimal modulation doses”** refer to the lowest concentration of a test compound required to modulate the nAChR channel activity.
 - **“Modulation”, “modulating” or “modulator”** of nAChR channel activity refers to the three distinct states that can be modulated and in which a nAChR channel can be configured: closed, open and desensitized. The binding of at least one ligand to the channel makes the transition between a closed (or a resting state) to an open (activated) state. Depending on the subunits constituting the nAChR channel the exposure time or the dose of the agonist (and optionally the co-agonist) the nAChR channel transits to a desensitized state. It also refers to

compounds including “agonists”, “antagonists”, “positive allosteric modulators”, “negative allosteric modulators”, “non-competitive channel blockers”, “open channel blockers” that may affect the current flow within the nAChR channel by inducing modification(s) of conformation on said nAChR channel, subunits assembly or organization, modification of the targeting, trafficking of the channels to the plasma membrane, modification of the life time of the said nAChR channel to the plasma membrane, or blocking the channel gate or any modification of post-translational state like phosphorylating state of the said nAChR channel.

- 10 - “**Nucleic acid sequence**” refers to encompass nucleic acids having the sequences set forth below as well as variants thereof including for example fragments, deletions, insertions and substitutions that maintain the ability to encode the different subunits of the nAChR channel of the invention.
- 15 - “**Phytosanitary product**” refers to biological or chemical compounds used as insecticides, herbicides, fungicides, fertilizers, antibiotics, or any products used in agriculture, in wine-making, food storage, ship bottom, for animals or domestic purposes.
- 20 - “**Sub-lethal doses**” refer to a concentration of a potentially lethal test compound that is not high enough to cause death meaning a concentration under the median lethal dose (LD₅₀) but still able to trigger death by a mechanism which is not acute (immediate). At sub-lethal dose, the test compound may induce changes in biological mechanisms that include but are not limited to: colony level behavioral changes, individual level behavioral changes, memory changes, cellular mechanisms or molecular mechanisms changes. The determination of mortality of some of these biological mechanisms changes is well-known in the state of the art. Therefore, technics determining such changes or mortality can easily be determined by the skilled artisan.
- 25 - “**Test compound**” refers to a phytosanitary product, a molecule, an organism or extract thereof eventually able to bind and/or modulate the nAChR channel activity of the invention.
- 30

- “**Variation**” refers to a single or several mutation(s) including end point mutation(s) or frameshift mutation(s), deletion(s), insertion(s), substitution(s), inversion(s), translocation(s), copy number loss, copy number gain.

5 DETAILED DESCRIPTION

One object of the present invention is the isolated nucleic acid sequences or a variant thereof comprising the subunits of the nAChR channel of a species from the phylum arthropoda.

The structure of the nAChR channel is well conserved between species. They belong to the cysteine-loop family of ligand-gated ion channels. In insects, nAChR channels are supposed to be constituted, by homology with vertebrates, by the assembly of 5 homologous subunits (pentameric structure) that form a membrane complex with an axial pseudo-symmetry and a central ion channel pore. In insects, 9 genes are encoding for α subunits (Am-nAChR α 1-9) while the β subunits are encoded by only two genes (Am-nAChR β 1-2). The functioning of the nAChR channel may sometimes require chaperone proteins (ric-3, unc-74, unc-50, emc-6). As with all ligand-gated ion channels, opening of the nAChR channel pore requires the binding of a chemical messenger. Several different terms are used to refer to the molecules that bind receptors, such as ligand.

The phylum arthropod is well-known from the skilled artisan that will recognize which species belong to this family. Arthropod phylum includes but is not limited to: the class of insects or arachnid.

The class of insects includes but is not limited to: pollinator insects, invasive insects.

Pollinator insects of the invention include species from the order *hymenoptera*, the family *Apidae* and the subfamily *Apinae* particularly the non-invasive species that does not damage crops or parasite other and/or native pollinator insects (insect native from a specific region), or damage hives.

Pollinator insects of the invention include in a non-limiting list: bees, honeybees such as *Apis mellifera*, *Apis cerana*, *Apis dorsata*, *Apis florea*, stingless bees such as *Melipona beecheii* or *Melipona yucatanica*, *Melipona quadrifasciata anthidioides*, orchid bees, bumble bees such as *Bombus franklini*, *Bombus terrestris*, *Bombus affinis* and *Bombus occidentalis*. Preferably, the pollinator insect of the invention is a honey bee, and most preferably *Apis mellifera*.

Invasive species of the invention include but are not limited to: introduced bees, imported bees, species from the order *hymenoptera*, the family *Vespidae* and the subfamily *Vespinae* particularly Asian predatory wasp (*Vespa velutina*), species from the class of arachnid.

The class of arachnid includes but is not limited to: the parasites, mites from the genus *Varroa*. The species of *Varroa* include but are not limited to: *Varroa destructor*, *Varroa jacobsoni*, *Varroa rindereri*, *Varroa sinhai*, *Varroa wongsirii*.

In one embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha1 subunit nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof consisting of a nucleic acid sequence having a maximum length of less than 2000; 1950; 1930; 1900; 1850 base pairs (bp) and having a minimum length of more than 1400; 1450; 1500; 1550; 1600; 1650; 1700; 1750 bp and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 1.

In one embodiment the variant of SEQ ID NO: 1 consists of a nucleic acid sequence of 1806 bp and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 1.

In one embodiment, a variation may occur in SEQ ID NO: 1.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha2 subunit nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof consisting of a nucleic acid sequence having a maximum length of less than 2000; 1950; 1930; 1900; 1850; 1800; 1750; 1700; 1650

base pairs (bp) and having a minimum length of more than 1300; 1400; 1450; 1500; 1550; 1600 bp and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 2.

In one embodiment, a variation may occur in SEQ ID NO: 2.

- 5 In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha3 subunit nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1702 base pair (bp), having a maximum length of less than 2500; 2400; 2300; 2200; 2100; 2000; 1900; 1850; 1800; 1750 bp and having at
10 least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 3.

In one embodiment, a variation may occur in SEQ ID NO: 3.

- In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha4 subunit nucleic acid sequence as set forth in
15 SEQ ID NO: 4 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1500; 1550; 1600; 1650; 1700 bp and having a maximum length of less than 1800; 1750; 1725 base pairs (bp) and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 4.

- 20 In one embodiment, a variation may occur in SEQ ID NO: 4.

- In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha5 subunit nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1000; 1100; 1200; 1300; 1350; 1400 bp and having a
25 maximum length of less than 1700; 1600; 1500; 1450 base pairs (bp) and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 5.

In one embodiment, a variation may occur in SEQ ID NO: 5.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha6 subunit nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof consisting of a nucleic acid sequence having a
5 minimum length of more than 1400; 1500; 1550; 1580; 1585 bp and having a maximum length of less than 1750; 1700; 1650; 1600 bp and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 6.

In one embodiment, a variation may occur in SEQ ID NO: 6.

10 In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha7 subunit nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof consisting of a nucleic acid sequence having a maximum length of less than 1800; 1750; 1740; 1700; 1650 base pairs (bp), having a minimum length of more than 1500; 1550; 1600; 1620; 1650 bp and having at least 80,
15 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 7.

In one embodiment, a variation may occur in SEQ ID NO: 7.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha8 subunit nucleic acid sequence as set forth in
20 SEQ ID NO: 8 or a variant thereof consisting of a nucleic acid sequence having a maximum length of less than 1900; 1950; 1800; 1850; 1800; 1750; 1700; 1650 base pairs (bp), having a minimum length of more than 1400; 1500; 1520; 1550; 1600 bp and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 97, 97.1, 97.2, 97.3, 97.4, 97.5, 97.6, 97.7, 97.8, 97.9, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1;
25 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 8.

In one embodiment, a variation may occur in SEQ ID NO: 8.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor alpha9 subunit nucleic acid sequence as set forth in

SEQ ID NO: 9 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1000; 1050; 1100; 1150; 1200; 1250 bp and having a maximum length of less than 1500; 1450; 1400; 1350; 1330; 1300 base pairs (bp) and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 9.

In one embodiment, a variation may occur in SEQ ID NO: 9.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor beta1 subunit nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1300; 1400; 1450; 1500; 1550 and having a maximum length of less than 1800; 1700; 1600; 1650; 1500; 1550 base pairs (bp) and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 10.

In one embodiment, a variation may occur in SEQ ID NO: 10.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the nicotinic acetylcholine receptor beta2 subunit nucleic acid sequence as set forth in SEQ ID NO: 11 or a variant thereof consisting of a nucleic acid sequence having a maximum length of less than 1500; 1450; 1400; 1350; 1300 base pairs (bp), having a minimum length of more than 1000; 1100; 1150; 1200; 1250 bp and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 11.

In one embodiment, the variant of SEQ ID NO: 11 consists in a nucleic acid sequence of 1284 base pairs (bp) and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.1, 98.2, 98.3, 98.4, 98.5, 98.6, 98.7, 98.8, 98.9, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 11.

In one embodiment, a variation may occur in SEQ ID NO: 11.

Another object of the present invention is the isolated nucleic acid sequences or a variant thereof comprising the chaperone protein of a species from the phylum arthropod.

- 5 In one embodiment of the invention, the isolated nucleic acid sequence comprises the chaperone protein resistance to inhibitors of cholinesterase 3 (ric-3) nucleic acid sequence as set forth in SEQ ID NO: 12 or variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1100; 1200; 1250; 1300; 1350 bp and having a maximum length of less than 2000; 1900; 1800; 1700; 1600; 1500; 1400 base
10 pairs (bp) and having at least 50, 60, 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 12.

In one embodiment, a variation may occur in SEQ ID NO: 12.

- In another embodiment of the invention, the isolated nucleic acid sequence comprises the chaperone protein endothelium reticulum (ER) membrane protein complex-6 (emc-
15 6) nucleic acid sequence as set forth as in SEQ ID NO: 13 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 100; 150; 200; 250; 300 bp and having a maximum length of less than 500; 400; 450; 300; 350 bp and having at least 50, 60, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 13.

- 20 In one embodiment, a variation may occur in SEQ ID NO: 13.

- In another embodiment of the invention, the isolated nucleic acid sequence comprises the chaperone protein unc-50 nucleic acid sequence as set forth as in SEQ ID NO: 14 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 500; 600; 650; 700; 750 bp and having a maximum length of less than 1000;
25 950; 900; 850 bp and having at least 50, 60, 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 14.

In one embodiment, a variation may occur in SEQ ID NO: 14.

In another embodiment of the invention, the isolated nucleic acid sequence comprises the chaperone protein unc-74 nucleic acid sequence as set forth as in SEQ ID NO: 15 or a variant thereof consisting of a nucleic acid sequence having a minimum length of more than 1000; 1050; 1100; 1150; 1200; 1250 bp and having a maximum length of less than 1600; 1550; 1500; 1450; 1400; 1350; 1300 bp and having at least 50, 60, 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 15.

In one embodiment, a variation may occur in SEQ ID NO: 15.

Another object of the present invention is a functional nicotinic acetylcholine receptor (nAChR) channel of a species from the phylum arthropod.

In one embodiment of the invention, the functional nAChR channel is from a pollinator insect.

In another embodiment of the invention, the functional nAChR channel is a parasite from the genus *Varroa*.

In one embodiment of the invention, the functional nAChR channel comprises at least one subunit of the nicotinic acetylcholine receptor (nAChR) channel.

In another embodiment of the invention, the functional nAChR channel comprises at least one subunit α of the nicotinic acetylcholine receptor (nAChR) channel.

In another embodiment of the invention, the functional nAChR channel comprises at least two identical subunits α of the nicotinic acetylcholine receptor (nAChR) channel.

In another embodiment of the invention, the functional nAChR channel does not comprise both subunits $\beta 1$ and $\beta 2$ of the nicotinic acetylcholine receptor (nAChR).

In one embodiment, the at least one subunit of the nicotinic acetylcholine receptor (nAChR) channel of the functional nAChR channel is phosphorylated.

In one embodiment, the at least one subunit of the nicotinic acetylcholine receptor (nAChR) channel of the functional nAChR channel undergoes post-translational modifications.

5 The assembly of the nAChR subunits, their expression on the cell surface, their interaction with the cytoskeleton, the open/resting, and desensitized state of the channel, the conductance of the channel can be affected by such post-translational modifications thereby modifying the channel functioning.

In another embodiment, the at least one subunit of the nicotinic acetylcholine receptor (nAChR) channel of the functional nAChR channel is palmitoylated.

10 In another embodiment, the at least one subunit of the nicotinic acetylcholine receptor (nAChR) channel of the functional nAChR channel is glycosylated.

In another embodiment, the at least one subunit of the nicotinic acetylcholine receptor (nAChR) channel of the functional nAChR channel is nitrosylated.

15 In one embodiment, a variation may occur on the nAChR subunits at the site of phosphorylation, palmitoylation, glycosylation, and/or nitrosylation.

Technics to induce variations such as deletion, mutation or insertion are well known in the state of the art and to the skilled artisan.

20 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof.

25 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

10 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as
15 set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

20 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor beta2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 11 or a variant thereof.

25 In one embodiment of the invention, the functional nAChR channel comprises at least one subunit of the nAChR channel and at least one of the chaperone proteins of a species from the phylum arthropod.

As with all ligand-gated ion channels, opening of the nAChR channel pore requires the binding of a chemical messenger. Several different terms are used to refer to the molecules that bind receptors, such as ligand.

5 In one embodiment, the functional nAChR channel of the invention is a functional selective cationic channel.

In one embodiment, said functional cationic channel is permeable for ions sodium (Na^+), potassium (K^+) or for some subunits calcium (Ca^{2+}).

10 In one embodiment, the functional nAChR channel of the invention comprises at least one subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 to 9 or a variant thereof.

15 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof.

20 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof.

25 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor

alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a

variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor

alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

5 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

10 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the
15 nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a
20 variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor
25 alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as

set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

- 5 In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the
10 nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

- In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a
15 variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

- In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor
20 alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

- In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor
25 alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a

variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof, the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor

alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a

variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor

alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

10 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the
15 nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a
20 variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor
25 alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as

set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

- 5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the
10 nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

- In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a
15 variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

- In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor
20 alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

- In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor
25 alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha2 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as

set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

10 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

15 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

20 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the
25 nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a

variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor

alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

10 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the
15 nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a
20 variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor
25 alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as

set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

- 5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the
10 nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

- In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha3 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a
15 variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

- In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor
20 alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

- In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor
25 alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor

alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a

variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor

alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

5 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

10 In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha4 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the
15 nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a
20 variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a
25 variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor

alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a

variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha5 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha6 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha7 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant thereof and the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha8 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof and In another embodiment, the functional nAChR channel of the invention comprises the nicotinic acetylcholine receptor alpha9 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises at least one subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 to 9 or a variant thereof and at least one chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 to 15 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises at least one subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 to 9 or a variant thereof and at least one subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 10 or 11 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises at least one subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 to 9 or a variant thereof, at least one subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 10 or 11 or a variant thereof and at least one chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 to 15 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 or a variant thereof.

5 In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

10 In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 1 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 5 or a variant thereof.

15 In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 5 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

20 In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 5 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 to 15 or a variant thereof.

25 In one embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 5 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 7 or

a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or
5 a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

10 In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 to 15 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the
15 subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or
20 a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the
25 subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 3 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof and the

chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 to 15 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 6 or
5 a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 13 to 15 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 6 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the
10 nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 9 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 9 or
15 a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 9 or
20 a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

In another embodiment, the functional nAChR channel of the invention comprises the subunit of nAChR encoded by the nucleic acid sequence consisting of SEQ ID NO: 9 or a variant thereof and the nicotinic acetylcholine receptor beta1 subunit encoded by the
25 nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof and the chaperone protein encoded by the nucleic acid sequence consisting of SEQ ID NO: 12 or a variant thereof.

Another object of the invention is an expression vector comprising at least one subunit of the nAChR channel of the invention or a variant thereof and able to express the channel in a suitable isolated cell transfected with said vector.

5 In one embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha1 subunit nucleic acid sequence as set forth in SEQ ID NO: 1 or a variant thereof.

In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha2 subunit nucleic acid sequence as set forth in SEQ ID NO: 2 or a variant thereof consisting of a nucleic acid sequence of less than 1930 base pairs (bp) and having at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 10 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 2.

In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha3 subunit nucleic acid sequence as set forth in SEQ ID NO: 3 or a variant thereof.

15 In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha4 subunit nucleic acid sequence as set forth in SEQ ID NO: 4 or a variant thereof.

In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha5 subunit nucleic acid sequence as set forth in SEQ ID NO: 5 or a variant thereof.

20 In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha6 subunit nucleic acid sequence as set forth in SEQ ID NO: 6 or a variant thereof.

In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha7 subunit nucleic acid sequence as set forth in SEQ ID NO: 7 or a variant 25 thereof.

In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha8 subunit nucleic acid sequence as set forth in SEQ ID NO: 8 or a variant thereof.

5 In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor alpha9 subunit nucleic acid sequence as set forth in SEQ ID NO: 9 or a variant thereof.

In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor beta1 subunit nucleic acid sequence as set forth in SEQ ID NO: 10 or a variant thereof.

10 In another embodiment, the expression vector comprises the nicotinic acetylcholine receptor beta2 subunit nucleic acid sequence as set forth in SEQ ID NO: 11 or a variant thereof.

Another object of the invention is an expression vector comprising at least one chaperone protein or a variant thereof of the invention and able to express said protein
15 in a suitable isolated cell transfected with said vector.

In another embodiment, the expression vector comprises the chaperone protein resistance to inhibitors of cholinesterase 3 (ric-3) nucleic acid sequence as set forth in SEQ ID NO: 12 or variant thereof.

20 In another embodiment, the expression vector comprises the chaperone protein ER membrane protein complex-6 (emc-6) nucleic acid sequence as set forth as in SEQ ID NO: 13 or a variant thereof.

In another embodiment, the expression vector comprises the chaperone protein unc-50 nucleic acid sequence as set forth as in SEQ ID NO: 14 or a variant thereof.

25 In another embodiment, the expression vector comprises the chaperone protein unc-74 nucleic acid sequence as set forth as in SEQ ID NO: 15 or a variant thereof.

Another object of the invention is an expression vector comprising at least one subunit of a nAChR channel or a variant thereof and at least one chaperone protein or a variant thereof of the invention and able to express said channel and protein in a suitable isolated cell transfected with said vector.

- 5 Another object of the invention is an isolated cell transfected with at least one vector described here above wherein said cell expresses the at least one vector described here above.

The isolated transfected cells as used herein refer to eukaryote cell. Isolated transformed cells are well known in the state of the art for genetic engineering. These cells include in
10 a non-limiting list: prokaryotic cells, eukaryotic cells, in particular bacteria such as *Escherichia coli*, *Bacillus sp.*, or yeasts such as *Saccharomyces cerevisiae*, fungus such as *Aspergillus niger*, insect cells such as SF9, SL1, or mammalian cells such as CHO, HEK293, PER-C6, amphibians cells such as for example *Xenopus* oocytes or oocytes of *Xenopus laevis*.

- 15 Technics for transfection or genetic engineering as used in the present application are well known by the person skilled in the art. These technics are described in Guide to Molecular Cloning Technics (Editors Berger SL and Kimmel AR 1987 Methods in Enzymology 152: 359-371).

Depending on the cell to be transfected, the person skilled in the art can determine the
20 technology needed for the introduction of the nucleotide sequences of the present application in the selected isolated cell to be transfected and the vector used.

Technics to transfect isolated cells which comprise introducing the nucleic acid molecules into the isolated cells may involve the use of expression vectors which comprise the nucleic acid molecules. These expression vectors (such as plasmids and
25 viruses; viruses including bacteriophage) can then be used to introduce the nucleic acid molecules into suitable isolated cells. For example, nAChR channel expression can be studied in *Xenopus* oocytes. DNA encoding the nAChR channel of the invention can be injected or transferred or transfected into the oocyte nucleus using a suitable vector, or

mRNA encoding the said nAChR channel can be injected directly into the oocyte, in order to obtain expression of a functional nAChR channel in the oocyte.

Various methods are known in the art for introducing nucleic acid molecules into isolated cells. One method is microinjection, in which DNA is injected directly into the nucleus of cells through fine glass needles (or RNA is injected directly into the cytoplasm of cells). Alternatively, DNA can be incubated with an inert carbohydrate polymer (dextran) to which a positively charged chemical group (DEAE, for diethylaminoethyl) has been coupled. The DNA sticks to the DEAE-dextran via its negatively charged phosphate groups. These large DNA-containing particles stick in turn to the surfaces of cells, which are thought to take them in by a process known as endocytosis. Some of the DNA evades destruction in the cytoplasm of the cell and escapes to the nucleus, where it can be transcribed into RNA like any other gene in the cell. In another method, cells efficiently take in DNA in the form of a precipitate with calcium phosphate. In electroporation, cells are placed in a solution containing DNA and subjected to a brief electrical pulse that causes holes to open transiently in their membranes. DNA enters through the holes directly into the cytoplasm, bypassing the endocytotic vesicles through which they pass in the DEAE-dextran and calcium phosphate procedures (passage through these vesicles may sometimes destroy or damage DNA). DNA can also be incorporated into artificial lipid vesicles, liposomes, which fuse with the cell membrane, delivering their contents directly into the cytoplasm. In an even more direct approach, used primarily with plant cells and tissues, DNA is absorbed to the surface of tungsten micro projectiles and fired into cells with a device resembling a shotgun.

Several methods of microinjection, electroporation, and liposome fusion, have been adapted to introduce nucleic acids into cells and are well known by the skilled artisan.

Further methods for introducing nucleic acid molecules into cells involve the use of viral vectors. Since viral growth depends on the ability to get the viral genome into cells, viruses have devised clever and efficient methods for doing it. One such virus widely used for protein production is an insect virus, baculovirus. Baculovirus attracted the attention of researchers because during infection, it produces one of its structural

proteins (the coat protein) to spectacular levels. If a foreign gene were to be substituted for this viral gene, it too ought to be produced at high level. Baculovirus, like vaccinia, is very large, and therefore foreign genes must be placed in the viral genome by recombination. To express a foreign gene in baculovirus, the gene of interest is cloned in place of the viral coat protein gene in a plasmid carrying a small portion of the viral genome. The recombinant plasmid is cotransfected into insect cells with wild-type baculovirus DNA. At a low frequency, the plasmid and viral DNAs recombine through homologous sequences, resulting in the insertion of the foreign gene into the viral genome. Virus plaques develop, and the plaques containing recombinant virus look different because they lack the coat protein. The plaques with recombinant virus are picked and expanded. This virus stock is then used to infect a fresh culture of insect cells, resulting in high expression of the foreign protein. Various viral vectors have also been used to transfect cells, such as bacteriophage, vaccinia virus, adenovirus, and retrovirus.

Another object of the invention is an isolated cell transfected with at least one vector comprising the acid nucleic sequences as described here above.

Another object of the present invention is an isolated cell expressing the functional channel of the invention comprising the isolated amino acid sequence of at least one subunit of the nAChR channel or a variant thereof.

Another object of the present invention is an isolated cell expressing the functional channel of the invention comprising the isolated amino acid sequence of at least one subunit of the nAChR channel or a variant thereof and at least one chaperone protein of a species from the phylum arthropod or a variant thereof.

In one embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha1 subunit amino acid sequence as set forth in SEQ ID NO: 16 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 16.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha2 subunit amino acid sequence as set forth in SEQ ID NO: 17 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of
5 identity with SEQ ID NO: 17.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha3 subunit amino acid sequence as set forth in SEQ ID NO: 18 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of
10 identity with SEQ ID NO: 18.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha4 subunit amino acid sequence as set forth in SEQ ID NO: 19 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of
15 identity with SEQ ID NO: 19.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha5 subunit amino acid sequence as set forth in SEQ ID NO: 20 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of
20 identity with SEQ ID NO: 20.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha6 subunit amino acid sequence as set forth in SEQ ID NO: 21 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of
25 identity with SEQ ID NO: 21.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha7 subunit amino acid sequence as set forth in SEQ ID NO: 22 or a variant thereof consisting of an amino acid sequence of at least 90, 91,

92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 22.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha8 subunit amino acid sequence as set forth in SEQ
5 ID NO: 23 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 23.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor alpha9 subunit amino acid sequence as set forth in SEQ
10 ID NO: 24 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 24.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor beta1 subunit amino acid sequence as set forth in SEQ
15 ID NO: 25 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 25.

In another embodiment of the invention, the isolated amino acid sequence comprises the nicotinic acetylcholine receptor beta2 subunit amino acid sequence as set forth in SEQ
20 ID NO: 26 or a variant thereof consisting of an amino acid sequence of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 26.

Another object of the present invention is the isolated amino acid sequences or a variant thereof comprising the chaperone protein of a species from the phylum arthropod.

25 In one embodiment of the invention, the isolated amino acid sequence comprises the chaperone protein resistance to inhibitors of cholinesterase 3 (ric-3) amino acid sequence as set forth in SEQ ID NO: 27, or a variant thereof consisting of an amino acid sequence having less than 480 bp and of at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89,

90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 27.

In another embodiment of the invention, the isolated amino acid sequence comprises the chaperone protein emc-6 amino acid sequence as set forth in SEQ ID NO: 28 or variant
5 thereof consisting of an amino acid sequence of at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 28.

In another embodiment of the invention, the isolated amino acid sequence comprises the chaperone protein unc-50 amino acid sequence as set forth in SEQ ID NO: 29 or variant
10 thereof consisting of an amino acid sequence of at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 29.

In another embodiment of the invention, the isolated amino acid sequence comprises the chaperone protein unc-74 amino acid sequence as set forth in SEQ ID NO: 30 or variant
15 thereof consisting of an amino acid sequence of at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1; 99.2; 99.3; 99.4; 99.5; 99.6; 99.7; 99.8; 99.9% of identity with SEQ ID NO: 30.

Another object of the invention is an *in vitro* method to determine the effect of a test compound on the activity of a nAChR channel of an arthropod comprising:

- 20 a. contacting an isolated cell described herein with at least one test compound,
 b. measuring the effect of said test compound on the nAChR channel activity, and

comparing said effect to the effect without test compound, thereby determining a
25 modulating activity of said nAChR channel.

Another object of the invention is an *in vitro* method to determine the effect of a test compound on the activity of a nAChR channel of an arthropod comprising:

- a. contacting an isolated cell described herein with at least one known nAChR channel modulator,

- b. measuring the effect of said modulator on the nAChR channel activity,
 - c. contacting said isolated cell described herein with at least one test compound and said at least one modulator,
 - d. measuring the effect of said modulator and test compound on the nAChR
- 5 channel activity, and

comparing the effect measured in d) to the effect measured in b), thereby determining a modulating activity of said nAChR channel.

Examples of known modulators of nAChR channel include but are not limited to: agonists, partial agonists, antagonists, allosteric modulators, positive allosteric

10 modulators, negative allosteric modulators, non-competitive channel blockers, open channel blockers of the nAChR channel of the invention.

Examples of agonists include but are not limited to: ACh, nicotine, epibatidine, choline, levamisole, carbachol, methyridine, Pysostigmine, Galantamine, Neostigmine, Pyridostigmine, Varenicline, Dimethylphenylpiperazinium (DMPP), Muscarine,

15 Oxotremorine, Bethanechol, Pilocarpine, clothianidin, Imidacloprid, acetamiprid, dinotefuran, derivate of nithiazine, nitenpyram, thiacloprid, thiamthoxan.

Examples of partial agonists include but are not limited to: acetylcholine binding protein (AChBP), benzoylcholine, GTS-21, choline.

Examples of antagonists include but are not limited to: α -bungarotoxin, dihydroxy- β -erythroidine, methyllycaconitine, α -conotoxin, α -tubocurarine, curare derivatives,

20 Pancuronium, Vecuronium, Atracurium, mecamylamine, suxamethonium, Trimethaphan, Mecamylamine, Bupropion, Dextromethophan, Hexamethonium, Atropine, Tolterodine, Vedaclidine, Talsaclidine, Xanomeline, Ipratropium, Pirenzepine, Telenzepine, Darifenacin, N-2-chloroethyl-4-piperidinyldiphenylacetate (4-DAMP),

25 Darifenacin, Solifenacin.

Examples of allosteric modulators include but are not limited to: neurosteroids.

Examples of positive allosteric modulators include but are not limited to: derivatives of (2-amino-5-keto)thiazole, desformylflustrabromine, galanthamine, codeine, serine, ivermectine.

5 Examples of negative allosteric modulators include but are not limited to: kyruneic acid, derivatives of methyllycaconitine.

Examples of non-competitive channel blockers include but are not limited to: piperidine derivative, mecamlamine, suxaméthonium, Trimethaphan, Mecamlamine, Bupropion, Dextromethophan, Hexamethonium.

10 In one embodiment of the invention, said known agonist of nAChR channel is applied for example from 1; 2; 3; 4; 5 ms to 10 seconds.

Another object of the invention is an *in vitro* method to determine the effect of a test compound on the activity of a nAChR channel of an arthropod comprising:

- a. contacting an isolated cell described herein with a standard reference,
- b. measuring the effect of said standard reference on the nAChR channel activity,
- 15 c. contacting said isolated cell described herein with at least one test compound and a standard reference,
- d. measuring the effect of said standard reference and said at least one test compound on the nAChR channel activity, and
- 20 comparing the effect measured in d) to the effect measured in b), thereby determining a modulating activity of said nAChR channel.

In one embodiment, the standard reference is a compound known to modulate the nAChR channel activity of the invention.

25 Examples of a standard reference include but are not limited to: known agonists, known partial agonists, known antagonists, known allosteric modulators, known positive allosteric modulators, known negative allosteric modulators, known non-competitive channel blockers, known open channel blockers of nAChR channel of the invention.

In another embodiment of the invention, the standard reference is a compound known to modulate the nAChR channel activity of other species such as invasive species. Examples of invasive species include but are not limited to: introduced species, imported bees, species from the order *hymenoptera*, the family *Vespidae* and the subfamily *Vespinae* particularly Asian predatory wasp (*Vespa velutina*), spiders, parasites, endoparasites, ectoparasites from the class *Arachnida*, the family *Varroidae* such as: *Varroa jacobsoni*, *Varroa destructor*, *Varroa underwoodi*, *Varroa rindereri*.

In another embodiment, the standard reference comprises at least one compound known to modulate the nAChR channel of the invention.

10 In one embodiment, lethal dose of a test compound is added to the culture medium.

In another embodiment, sub-lethal dose of a test compound is added to the culture medium.

In another embodiment, minimal modulation dose of a test compound is added to the culture medium.

15 In another embodiment, the test compound is not toxic for pollinator insects and toxic for invasive species.

In another embodiment, the test compound is not toxic for *Apis mellifera* and toxic for *varroa*.

20 Examples of test compounds comprise phytosanitary product which include but are not limited to: insecticides, pesticides, drugs, veterinary drugs (such as veterinary drugs against parasites) that include in a non-limiting list derivatives or analogs of: neonicotinoid family compounds, acetamiprid, clothianidin (Poncho®), dinotefuran, imidacloprid (Gaucho®), derivate of nithiazine, nitenpyram, thiacloprid, and thiamethoxan (Cruiser®).

25 Examples of test compounds also comprise but are not limited to compounds already known to be toxic on other species such as invasive species as described here above.

Examples of test compounds comprise molecules which include but are not limited to: siRNAs, shRNAs, antisense oligonucleotide, ribozymes or aptamers, ligands, agonist or antagonist of nAChR, antibodies or fragments thereof, diabodies modulating activity of a nAChR of a pollinator insect. These test compounds can also include derivatives, analogs of: neurotoxins such as snake venoms α -neurotoxins, toxins from plants, venom of insects, spiders, cones, mollusks, snails, and vertebrates such as snakes, scorpion toxins, or any other natural products used in integrated pest management.

In one embodiment of the invention, said test compound is already known to modulate the nAChR channel activity.

10 In another embodiment of the invention, said test compound is not yet known to modulate the nAChR channel activity.

In another embodiment of the invention, said test compound is a new chemical entity.

In one embodiment of the invention, said test compound increases or decreases the functional expression of nAChR channel in the isolated transfected cell.

15 In one embodiment of the invention, said test compound induces or reduces the nAChR channel expression at the cell surface.

In one embodiment, said test compound modulates nAChR channel activity via $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 8$, and/or $\alpha 9$ subunit.

In one embodiment, said test compound modulates nAChR channel activity via $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 8$, and/or $\alpha 9$ subunit and the chaperone protein.

In another embodiment, said test compound modulates nAChR channel activity via $\beta 1$ or $\beta 2$ subunit.

In another embodiment, said test compound modulates nAChR channel activity via $\beta 1$ or $\beta 2$ subunit and the chaperone protein.

25 In another embodiment, said test compound modulates nAChR channel activity via $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 8$, or $\alpha 9$ subunit and $\beta 1$ and/or $\beta 2$ subunit.

In another embodiment, said test compound modulates nAChR channel activity via $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 8$, or $\alpha 9$ subunit and $\beta 1$ and/or $\beta 2$ subunit and the chaperone protein.

5 nAChRs may exist in different interconvertible conformational states. Binding of an agonist stabilizes the open and desensitized states. Opening of the channel allows positively charged ions to move across it; in particular, sodium enters the cell and potassium exits. The net flow of positively-charged ions is inward.

10 The nAChR is a selective cation channel, meaning that several different positively charged ions can cross through. It is permeable to Na^+ and K^+ , with some subunit combinations that are also permeable to Ca^{2+} .

In one embodiment, said test compound modulates the activity of the nAChR channel of the invention.

In one embodiment, said test compound modifies the gating kinetics of the nAChR channel.

15 In another embodiment, said test compound modulates (increases or decreases) the hyperpolarization due to the activity of the nAChR channel of the invention.

In another embodiment, said test compound modulates (increases or decreases) the depolarization due to the activity of the nAChR channel of the invention.

20 In another embodiment, said test compound modulates (increases or decreases) the ion uptake by the nAChR channel of the invention.

In another embodiment, said test compound induces deleterious neuronal hyperexcitability.

In another embodiment, said test compound modulates (increases or decreases) the duration of closed states.

25 In another embodiment, said test compound modulates (increases or decreases) the duration of open states.

In another embodiment, said test compound modulates (increases or decreases) the activation and/or desensitization kinetics of the nAChR channel of the invention.

In another embodiment, said test compound affinity is modulated upon channel opening.

- 5 In another embodiment, said test compound affinity is modulated upon channel closed-state.

In another embodiment, said test compound is dependent of the functional states of the nAChR channel with preferred affinity for the close and/or open and/or desensitized states.

- 10 In another embodiment, said test compound modulates (increases or decreases) the sorting, the targeting or the translocation of the nAChR channel of the invention.

In another embodiment, said test compound modulates (increases or decreases) the stability of the nAChR channel of the invention.

- 15 In another embodiment, said test compound modulates the subunits assembly of the nAChR channel of the invention.

In one embodiment, the method of the invention is a cell-based assay.

In one embodiment, the method of the invention is a high-throughput assay.

In another embodiment, the method of the invention is an electrophysiological method.

- 20 In another embodiment, the method of the invention is a fluorometry or luminometry method.

The methods of the invention can be in conventional laboratory format or adapted for high throughput. The term “high throughput” (HTS) refers to an assay design that allows easy analysis of multiple samples simultaneously, and capacity for robotic manipulation. Another desired feature of high throughput assays is an assay design that

is optimized to reduce reagent usage, or minimize the number of manipulations in order to achieve the analysis desired.

Methods for measuring the effect of a test compound on a nAChR channel are well known in the state of the art. For example, the person skilled in the art knows that
5 electrophysiological measurements in isolated transfected cells expressing functional nAChR channel can be used to test a compound.

In one embodiment, measuring the effect of a test compound comprises measuring the activation kinetics of the nAChR channel of the invention.

In another embodiment, measuring the effect of a test compound comprises measuring
10 the deactivation kinetics of the nAChR channel during removal of the agonist.

In another embodiment, measuring the effect of a test compound comprises measuring the desensitization kinetics of the nAChR channel of the invention.

In another embodiment, measuring the effect of a test compound comprises measuring the current amplitude of the nAChR channel of the invention.

15 In another embodiment of the invention, the *in vitro* method can be used to screen a compound that inhibits, prevents or stops the action of a compound known to intoxicate an arthropod through its nAChR channel subunits.

In one embodiment of the invention, the *in vitro* method determine the effect of a test compound to provide modulation reference patterns and databases of modulation
20 reference patterns for a wide range of molecules. The reference patterns are then used for the identification and classification of test molecules. Evaluation of test compounds may be used to achieve different results.

Methods for the classification of compounds according to the spectral density signature of evoked changes in cellular electric potential are known to the person skilled in the
25 art; see, e.g., US patent No 6,377,057. Thus, compounds are classified according to their effect on ion channels, changes in membrane potential and ionic currents, and the frequency content of action potentials that the compound(s) evoke in excitable cells.

The spectral density changes of such evoked membrane potential or action potential are a characteristic for each channel type that is modulated by the test compound. A pattern of spectral changes in membrane potential is determined by contacting a responsive cell with a test compound, and monitoring the membrane potential or ionic currents over
5 time. These changes correlate with the effect of that compound, or class of compounds, on the ion channels of the responding cell. This pattern of spectral changes provides a unique signature for the compound, and provides a useful method for characterization of channel modulating agents. The effect of a compound on ion channels, and on the action potential of a living cell, can provide useful information about the classification
10 and identity of the compound. Methods and means for extracting such information are of particular interest for the analysis of molecules, with specific applications in pharmaceutical screening, drug discovery, environmental monitoring, biowarfare detection and classification, and the like. Examples of whole cell-based biosensors are described in Gross et al., Biosensors and Bioelectronics 10 (1995), 553-567.

15 Another object of the invention is an *in vitro* method to determine the toxicity of a test compound on an arthropod comprising:

- a. contacting an isolated cell described herein with at least one test compound,
 - b. measuring the effect of said at least one test compound on the nAChR channel activity, and
- 20 comparing the effect to the effect without test compound, thereby determining the toxicity on said nAChR channel.

In one embodiment of the invention, a test compound is considered as toxic once a modulating effect is measured on the nAChR channel of the invention.

In one embodiment, a test compound is considered as toxic once it modifies the gating
25 kinetics of the nAChR channel of the invention.

In another embodiment, a test compound is considered as toxic once it modulates (increases or decreases) the amplitude of ion current of the nAChR channel of the invention.

In another embodiment, a test compound is considered as toxic once it modulates (increases or decreases) the desensitized state.

In another embodiment, a test compound is considered as toxic once it alters the activation kinetics or voltage dependence of either channel activation or desensitization.

- 5 In another embodiment, a test compound is considered as toxic once it induces deleterious neuronal hyperexcitability.

In another embodiment, a test compound is considered as toxic once its affinity is modulated (increased or decreased) upon channel opening.

- 10 In another embodiment, a test compound is considered as toxic once it modulated channel closed-state.

In another embodiment, a test compound is considered as toxic once it is dependent of the functional states of the nAChR channel with preferred affinity for the close and/or open and/or desensitized states.

- 15 In another embodiment, a test compound is considered as toxic once the sorting, the targeting or the translocation of the nAChR channel of the invention is modified.

In another embodiment, a test compound is considered as toxic once the stability of the nAChR channel of the invention is modified.

In another embodiment, a test compound is considered as toxic once the subunits assembly of nAChR channel of the invention is modified.

- 20 In one embodiment, the test compound is not toxic for the pollinator insects of the invention.

In another embodiment, the test compound is toxic for the pollinator insects of the invention.

- 25 In another embodiment, the test compound is toxic for invasive species while said test compound is not toxic for the pollinator insects of the invention.

In another embodiment, the test compound is toxic for insects spreading diseases to a human subject. Examples of such insects are mosquitoes. Examples of such diseases include but are not limited to: malaria, dengue fever, Japanese encephalitis, Ross River virus infection, Barmah Forest virus infection, Murray Valley encephalitis, yellow fever, West Nile Virus.

Another object of the invention is an *in vitro* method for screening compounds that modulate the nAChR channel activity of an arthropod that comprises:

- a. contacting an isolated cell described herein with at least one test compound,
 - b. measuring the effect of said at least one test compound on the nAChR channel activity, and
- comparing said effect to the effect without test compound, thereby determining a modulation of activity of said nAChR channel and classifying said compound as an agonist, a partial agonist, antagonist, positive allosteric modulator, negative allosteric modulator, non-competitive channel blocker, open channel blocker.

Another object of the invention is an *in vitro* method for screening compounds that modulate the nAChR channel activity of an arthropod that comprises:

- a. contacting an isolated cell described herein with at least one agonist,
- b. measuring the effect of said agonist on the nAChR channel activity, and
- c. contacting the isolated cell described herein with said agonist compound and at least one test compound,
- d. measuring the effect of said agonist and said test compound on the nAChR channel activity, and

comparing the effect measured in d) to the effect measured in b), thereby determining a modulation of activity of said nAChR channel and classifying said compound as an agonist, a partial agonist, antagonist, positive allosteric modulator, negative allosteric modulator, non-competitive channel blocker, open channel blocker.

Another object of the invention is an *in vitro* method to detect the binding of a test compound on the nAChR channel of an arthropod comprising:

- a. contacting an isolated cell described herein with at least one test compound,
- and

- b. measuring the binding of said test compound on the nAChR channel of a pollinator insect.

In one embodiment, the method of the invention determines the ability of a test compound to bind to the nAChR channel of the invention.

- 5 Tests to monitor binding of a ligand to the nAChR channel of the invention are well known in the state of the art. Such tests include but are not limited to: radioligand binding test ($[^3\text{H}]$ -epibatidine and $[^{125}\text{I}]$ - α -bungarotoxine).

Another object of the invention is a kit comprising the vector of the invention or the isolated cell expressing the nAChR channel of the invention and reagents for
10 conducting any one of the above described methods of the invention.

Optionally the kit can comprise culture medium, recombinant nucleic acid sequences, reagents, standard reference compounds, etc. Such kit would typically comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents useful for performing said methods. The
15 carrier may also contain a means for detection such as labeled enzyme substrates or the like. Instructions can be provided to detail the use of the components of the kit, such as written instructions, video presentations, or instructions in a format that can be opened on a computer (e.g. a diskette or CD-ROM disk). These instructions indicate, for example, how to use the cells to screen test compounds of interest (such as ionotropic
20 drugs).

In addition, the present invention relates to an apparatus and array, respectively, for use in the methods and assays of the present invention described herein. For example, a cell-potential measurement apparatus having a plurality of microelectrodes and which may be used and/or adapted in accordance with the teaching of the present invention is
25 described in European patent application EP 0 689 051.

Furthermore, international application WO 98/54294 describes an apparatus and method for monitoring cells and a method for monitoring changes in cells upon addition of a compound to the cell's environment, comprising a device which includes an array of

microelectrodes disposed in a cell culture chamber, upon which array a portion of cells adhere to the surfaces of the microelectrodes. The diameter of the cells is larger than the diameters of the microelectrodes. A voltage signal is applied across each of the microelectrodes and a reference electrode. Detection and monitoring of the signals resulting from the application of the voltage signal provides information regarding the electrical characteristics of the individual cells, including impedance (combined cell membrane capacitance and conductance), action potential parameters, cell membrane capacitance, cell membrane conductance, and cell/substrate seal resistance.

The present invention also relates to an automated Voltage-Clamp Screening System for *Xenopus* oocytes comprising HiClamp robot, USB video camera, HiClamp software, accessories, and consumables which is a well-known technique in the state of the art.

The HiClamp is a fully-automated all-in-one solution for high-throughput functional secondary screening of test compounds based on the standard *Xenopus* expression system. The HiClamp functionality is based on the use of a novel system in which the oocyte is exposed to a test solution by moving it physically into the solution of interest, whereas in a standard system the solution is applied on the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents photographs representing tissue expression of Am-nAChR chaperones in *Apis Mellifera* (A: Antenna, L: leg, T: Thorax, B: Brain). These chaperone proteins are expressed ubiquitously in excitable muscles and neurons.

Figure 2 represents histograms (A) and current traces recorded (B) on oocytes showing the role of chaperone proteins ric3, unc74, unc50 and emc6 in the functional expression of two types of nAChR composed of different α subunits.

Figure 3 represents ACh dose-response curves for different combinations of nAChR subunits producing functional receptors. A-B. Current traces recorded on oocytes expressing the nAChR α 5 (A, with unc74+unc50+emc6) or the nAChR α 7 (B, with ric3) subunits in response to different concentrations of ACh. C. Dose-response curves for

ACh on the combination of subunits containing the nAChR α 5 or the nAChR α 7 subunit. Note the higher sensitivity of the receptors containing the nAChR α 7.

Figure 4 represents pharmacological sensitivity of honeybee nicotinic receptors Am-nAChR α 5 and Am-nAChR α 7. A. Current traces showing the effects of ACh and clothianidin (Poncho®, Bayer) at 500 μ M on the two types of receptors. B. Dose-response curves of clothianidin on these two types of receptors. C. Dose-response curves of ACh and clothianidin on Am-nAChR α 7 recorded on the same oocyte demonstrating that clothianidin is a less effective agonist than ACh. Please note the differential sensitivity of these two types of receptors, Am-nAChR α 5 being completely insensitive to clothianidin.

Figure 5 is a 3D histogram showing the expression of different combinations of *Apis mellifera* nicotinic acetylcholine receptor subunits. A. Current traces recorded from oocytes expressing the *Apis mellifera* α 1, α 3+Am- β 1, α 5+ceRic3 or α 7+ceRic3 combinations of nicotinic acetylcholine (ACh) receptor subunits and chaperone proteins. The perfusion of ACh (500 μ M, 3 s) is noted as a black line. The holding potential was -60 mV. Note the decrease in the inward current obtained when ACh was perfused on oocytes expressing the α 3 subunit.

B. 3D bar graph showing the averaged currents recorded on oocytes expressing different combination of nicotinic ACh receptor subunits and chaperone proteins. In these conditions a good expression was only recorded with combinations containing the α 3, α 5 and α 7 subunits.

Figure 6 represents of the voltage-dependence of the α 3 receptors. ACh (400 μ M) was applied on oocytes injected with the mentioned receptor/chaperone subunit compositions during 2 seconds at various membrane potentials (from -100 mV to -30 mV) and the corresponding ACh-induced current was recorded. Please, note that (1) ACh-induced current was outward relative to the holding current and (2) that the reversal potential of the ACh current was close to -30 mV.

Figure 7 represents the dose response curve of α 3 nACh receptors. A. Superimposed current traces recorded during the perfusion of various concentrations of ACh on oocyte

injected with the *Apis mellifera* $\alpha 3$ subunit co-expressed with the nACh receptor $\beta 2$ subunit. Note that the response was inward for low concentration of ACh and started to be outward for dose of ACh greater than 250 μ M. B. Full dose response curve of the effects of ACh measured as the peak inward current on Am- $\alpha 3$ + $\beta 2$ nACh receptor.

- 5 The EC_{50} and Hill values for the increasing effects (increase in peak inward current) were 5 μ M and 1.9, while the EC_{50} and Hill values for the decrease of the peak inward current were 79 μ M and 2.9.

Figure 8 represents superimposed effects of ACh (500 μ M) or the neonicotinoid clothianidin (500 μ M) on the Am- $\alpha 3$ + $\beta 2$ nACh receptor. Note that clothianidin
10 produced on averaged around 80% of the ACh response (see bar graph). The holding potential was -60 mV.

EXAMPLES

The present invention is further illustrated by the following examples.

- 15 Example 1: Cloning, sequencing and characterization of the nAChR subunits $\alpha 1$ -9 and nAChR $\beta 1$ -2, and the chaperone proteins ric3, emc-6, unc50 and unc74

The structure of the nAChR is well conserved between species. They belong to the cysteine-loop family of ligand-gated ion channels. In insects they are supposed to be constituted, by homology with vertebrates, by the assembly of 5 homologous subunits
20 (pentameric structure) that form a membrane complex with an axial pseudo-symmetry and a central ion channel pore. Binding of acetylcholine to the receptor produces a rapid opening of a pore able to select small cations for entry. Each subunit has 4 trans-membrane segments, M1-M4, with the M2 segment being constitutive of the channel pore, and a large extracellular N-terminal region forming a loop thanks to a disulfide
25 bridge between two conserved cysteines distant by 13 amino-acids. The N-terminal region of two adjacent subunits forms the ACh binding-pocket and participates, with the M3-M4 helices to the assembly and the kinetics of the receptors. The subunits that possess this dicystein loop are called α subunits, while those without are called non- α or β subunits. In honeybee, 9 genes are encoding for α subunits ($\alpha 1$ -9) while the β

subunits are encoded by only two genes. Thus, honeybee, like other insects (nAChR are cloned in 6 insect species) possesses a relatively small family (11 genes) of nAChR compare to vertebrates (15 to 30 genes) (Jones & Sattelle, 2006 Genome Res. 16, 1422-1430; Whitfield et al., 2002 Genome Res. 12, 555-566). Which subunit(s) form the functional nAChR that can be activated in different bee neurons is actually poorly known (but see (Dupuis et al., 2011 J Neurophysiol. 106, 1604-1613). If the sequence of each of these honeybee subunits has been isolated, their functional characterization in expression system such as *Xenopus* oocytes (or cell lines) has never been done. In other insects also, expression of nicotinic receptors has been done only using heteromers that include mammalian subunits, and receptors containing only insects subunits are reported to express at very low level only.

Using sequence homology with drosophila, the 11 genes coding for honeybee nAChR subunits have been classified in 7 groups, with one group (including the $\alpha 5$ - $\alpha 6$ - $\alpha 7$ subunits) beholding a common ancestors with the vertebrate subunits sensitive to bungarotoxine, while the 6 other groups ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 8$, $\beta 1$) have a distinct ancestor. The $\alpha 9$ and $\beta 2$ subunits are clearly distinct from these 7 groups. Subunits can oligomerize in oligo- or hetero-pentamers. These combinations, as well as of alternative splicing and RNA editing produce, despite the small number of genes, a very wide diversity of the receptors with different functional properties.

In bee brain these genes are expressed in the structures responsible for the processing of sensory, visual and olfactory information including the optic lobes, antennal neurons, the antennal lobes, and the mushroom bodies.

In vertebrate, bungarotoxine-sensitive (mostly homo-pentamers) and -insensitive (mostly heteropentamers) receptors have been distinguished. In insects, the fact that these subunits cannot be expressed without vertebrate subunits makes this distinction hard to make. However, heteromeric channels sensitive and insensitive to bungarotoxine have been detected. These two types of sensitivity can also be found in native neurons, but these studies are still very sparse. Imidaclopride (Gaucho®) and 6 of its derivatives have an important potency as nAChR agonist and a good selectivity for insect receptors.

Up to now, no honeybee nAChR have been expressed in a heterologous expression system to perform a precise characterization of the different subunits combinations. However, it has recently been shown, in *C-elegans*, but also in insects and in vertebrates, that other transmembrane proteins (from intracellular membrane) such as
5 ric3, unc50, unc74 or emc-6 may play an important role for the targeting and the recycling of the nAChR at the membrane.

Our analysis of the *Apis mellifera* genome identified 11 genes for α (α 1-9) and β (β 1 and β 2) subunits of nAChR. Sequence homology among these different subunits are between 25 to 70% as a function of their belonging to a specific group. These sequences
10 are ~99% homologous to those identified from the annotated genome of *Apis mellifera* in the public databases (Jones et al., 2006; Elsik et al., BMC Genomics. 2014 Jan 30;15:86). We have also identified and cloned in *Apis mellifera* 4 chaperone proteins homologous to the ric3, unc50, unc74 and emc-6 proteins of *C-elegans*. These honeybee chaperones show 13, 38, 40 and 30% homology with their homologues in *C-elegans*,
15 respectively, and 23% of homology are calculated between the honeybee and the drosophila ric3, the only chaperone isolated in drosophila. None of these chaperones was identified in honeybee before this work.

We have cloned, sequenced and characterized the nAChR subunits α 1-9 and nAChR subunits β 1-2. We have also cloned honeybee ric3, emc-6, unc50 and unc74 and
20 analyze their homology with *C-elegans* and drosophila subunits. We demonstrate expression of these subunits in different bee muscular and nervous tissues (such as *Apis mellifera* tissues, **Figure 1**).

Example 2: Pharmacology and sensitivity of nAChR channel in combination with chaperone protein to insecticides

25 We also show that functional expression in *Xenopus laevis* oocytes of these subunits are under the control of the chaperones proteins in the nAChR subunit-specific manner (**Figure 2**). For example, ric3 (from *C.elegans*, the nucleic acid sequence being SEQ ID NO: 64 and the amino acid sequence being SEQ ID NO: 65, or honeybee) is clearly necessary to get a reliable expression of the nAChR α 7 subunit and a response to ACh,

while for the nAChR α 5 subunit a cocktail of emc6, unc50 and unc74 chaperone proteins improves expression with response amplitude between 0.1 to several μ A for ACh concentration between 1 to 1000 μ M (**Figure 2**). In the case of the nAChR α 3 subunits, expression can be obtained either alone, or in combination with different cocktails of chaperone or with the vertebrate β 2 nAChR subunits. Each of these combinations has a specific affinity for ACh. Finally using the α 5 and α 7 subunits we show by constructing dose-response curves the specific response of each of these subunits to ACh (**Figure 3**). These two subunits have also a specific pharmacology and sensitivity to insecticides, with the α 5 subunit being insensitive to clothianidin (the active substance of Poncho®), while the α 7 subunit was half-activated for doses close to 10 μ M (**Figure 4**). In addition, expressing in oocytes the *Apis mellifera* α 1, α 3+Am- β 1, α 5+ceRic3 or α 7+ceRic3 combinations of nicotinic ACh receptor subunits and chaperone proteins allowed to record ACh-induced current (**Figure 5A**). These data demonstrate that toxicological tests need to be realized on more than one combination of nAChR subunits to be relevant.

Indeed, **Figure 5B** shows diverse combinations of *Apis mellifera* nicotinic acetylcholine receptor subunits responding to ACh.

More specifically, combining the expression of the subunit α 3 and the chaperone proteins unc50 + emc60 shows a response to 400 μ M of ACh that is the opposite of the normal response, but still reverses and the normal reversal potential (**Figure 6**). Moreover, combining the expression of the subunit α 3 and β 2 shows a dose-response curve to ACh that is well-shaped with two distinct EC50 for the increasing and decreasing parts of the curve (**Figure 7 A - B**). **Figure 8** shows that such combination is not only sensitive to ACh but also to clothianidin.

The use of this technology (perfusion of different chemical agents on different combination of nAChR subunits identified on honeybee neuron or muscle) may therefore bring precise and important information on the toxicity of these products on bees (as for example *Apis mellifera*, but also *Apis cerana*, *Apis dorsata* or *Apis florea*).

- To our knowledge, while the nAChR sequences are known (Jones et al., 2006, Elsik et al., 2014), no heterologous expression of these subunits have been reported. Moreover, while the genes coding for *ric3*, *emc-6*, *unc50* and *unc74* have been functionally characterized in *C-elegans* and shown to play a role in nAChR expression (Boulin et al.,
5 Proc Natl Acad Sci U S A. 2008 Nov 25; 105(47): 18590–18595), no data are available on the role of their homologues in honeybee. As a consequence, up to now, no cellular model were available to test the toxicity of environmental stressor on bee nAChR without the use of either whole animals (adult or larva), or primary culture of muscle cells or neurons.
- 10 The fact that different combinations of *Apis mellifera* nAChR subunits can now be expressed in heterologous system and used to test the toxicity of chemical opens the way to more standardized and automated methods to set-up toxicological tests but also for differential screening new insecticide safe for pollinators.

Materials and Methods

15 Total RNAs purification from bees

- Bees have been anesthetized at 4°C. Whole brains, legs, antennas and abdomens, have been rapidly dissected under a binocular and then stored on ice. Total RNAs have been purified from whole brains with the RNeasy Mini Kit (Qiagen). For the other tissues and for larva (Day7 and Day18), total RNAs have been purified with the RNAwiz reagent (Life Technologies). The tissues have been homogenized in 600 µl of RLT
20 Buffer (Qiagen) or 4ml of RNAwiz (Life Technologies). The remaining of the procedure has been carried on following the instructions of the manufacturers. The final concentration of total RNAs have been determined by using a spectrophotometer (Biophotometer, Eppendorf), and RNA integrity has been checked by running an aliquot
25 of RNA on an agarose gel. Total RNAs have then been stored at -80°C until use.

Reverse transcription and PCR amplification

The first strand of the cDNAs has been obtained with the SuperScript II Reverse Transcriptase (Life Technologies) and with Oligo(dT)18 primers 1 µl of the obtained

cDNAs has been subjected to PCR amplification with the Herculase II fusion polymerase (Agilent Technologies). The temperature and the duration of the denaturation step (92-98°C, 20-60s), of the hybridization step (55-65°C, 20-60s) and of the extension step (68 or 72°C, 30s-3mn), together with the final concentration of DMSO (0 to 8%) of the PCR reactions have been empirically optimized for each couple of primers. The PCRs displayed on the Figure 1 (Am-ric3, Am-unc50, Am-unc74, Am-emc6 have been obtained with the following couple of primers amric3-008S and amric3-005AS, amunc50-001S and amunc50-002AS, amunc74-001S and amunc74-002AS and amemc6-001S and amemc6-002AS, respectively (Table 1). When appropriate, amplified fragments have been purified from agarose gel with the NucleoSpin Extract II kit (Macherey-Nagel), phosphorylated with the T4 kinase (Life Technologies) and ligated into the expression vector pCMV-PL10 which is a modified version of pcDNA3.1(+) (Life Technologies) with the sequence of the Alfalfa Mosaic Virus (AMV) immediately before the start codon and the 3'-UTR sequence of the Xenopus β -globin gene after the stop codon to boost expression in Xenopus oocytes. Recombinant plasmids have been sequenced on both strands by Eurofins MWG Operon.

Amplification of the sequence of the Am-nAChR α 1 subunit

A PCR carried out using the primer amnachra1-001S (SEQ ID NO: 31) and amnachra1-002AS (SEQ ID NO: 32) has allowed the identification of the nucleotides 1 to 1806 (SEQ ID NO: 1).

Amplification of the sequence of the Am-nAChR α 2 subunit

A PCR carried out using the primer amnachra2-001S (SEQ ID NO: 33) and amnachra2-002AS (SEQ ID NO: 34) has allowed the identification of the nucleotides 1 to 1626 (SEQ ID NO: 2).

Amplification of the sequence of the Am-nAChR α 3 subunit

A PCR carried out using the primer amnachra3-001S (SEQ ID NO: 35) and amnachra3-002AS (SEQ ID NO: 36) has allowed the identification of the nucleotides 1 to 1704 (SEQ ID NO: 3).

5 Amplification of the sequence of the Am-nAChR α 4 subunit

A PCR carried out using the primer amnachra4-001S (SEQ ID NO: 37) and amnachra4-002AS (SEQ ID NO: 38) has allowed the identification of the nucleotides 1 to 1710 (SEQ ID NO: 4).

Amplification of the sequence of the Am-nAChR α 5 subunit

- 10 A PCR carried out using the primer amnachra5-003S (SEQ ID NO: 39) and amnachra5-002AS (SEQ ID NO: 40) has allowed the identification of the nucleotides 1 to 1446 (SEQ ID NO: 5).

Amplification of the sequence of the Am-nAChR α 6 subunit

- 15 A PCR carried out using the primer amnachra6-001S (SEQ ID NO: 41) and amnachra6-002AS (SEQ ID NO: 42) has allowed the identification of the nucleotides 1 to 1590 (SEQ ID NO: 6).

Amplification of the sequence of the Am-nAChR α 7 subunit

- 20 A PCR carried out using the primer amnachra7-001S (SEQ ID NO: 43) and amnachra7-002AS (SEQ ID NO: 44) has allowed the identification of the nucleotides 1 to 1668 (SEQ ID NO: 7).

Amplification of the sequence of the Am-nAChR α 8 subunit

A PCR carried out using the primer amnachra8-001S (SEQ ID NO: 45) and amnachra8-002AS (SEQ ID NO: 46) has allowed the identification of the nucleotides 1 to 1614 (SEQ ID NO: 8).

Amplification of the sequence of the Am-nAChR α 9 subunit

A PCR carried out using the primer amnachra9-001S (SEQ ID NO: 47) and amnachra9-002AS (SEQ ID NO: 48) has allowed the identification of the nucleotides 1 to 1296 (SEQ ID NO: 9).

5 Amplification of the sequence of the Am-nAChR β 1 subunit

A PCR carried out using the primer amnachrb1-001S (SEQ ID NO: 49) and amnachrb1-002AS (SEQ ID NO: 50) has allowed the identification of the nucleotides 1 to 1563 (SEQ ID NO: 10).

Amplification of the sequence of the Am-nAChR β 2 subunit

- 10 A PCR carried out using the primer amnachrb2-001S (SEQ ID NO: 51) and amnachrb2-007AS (SEQ ID NO: 52) has allowed the identification of the nucleotides 1 to 1284 (SEQ ID NO: 11).

Amplification of the sequence of Cele ric3

- 15 A PCR carried out using the primer ceric3-001S (SEQ ID NO: 53) and ceric3-002AS (SEQ ID NO: 54) has allowed the identification of the nucleotides 1 to 1137 (SEQ ID NO: 64)

Amplification of the sequence of am ric3a

- 20 A PCR carried out using the primer amric3-008S (SEQ ID NO: 55) and amric3-007AS (SEQ ID NO: 63) has allowed the identification of the nucleotides 1 to 1365 (SEQ ID NO: 12).

Amplification of the sequence of am emc6

A PCR carried out using the primer emc6-001S (SEQ ID NO: 57) and emc6-002AS (SEQ ID NO: 58) has allowed the identification of the nucleotides 1 to 342 (SEQ ID NO: 13).

Amplification of the sequence of am unc50

A PCR carried out using the primer amunc50-001S (SEQ ID NO: 59) and amunc50-002AS (SEQ ID NO: 60) has allowed the identification of the nucleotides 1 to 804 (SEQ ID NO: 14).

5 Amplification of the sequence of am unc74

A PCR carried out using the primer amunc74-001S (SEQ ID NO: 61) and amunc74-002AS (SEQ ID NO: 62) has allowed the identification of the nucleotides 1 to 1296 (SEQ ID NO: 15).

Oligonucleotides/primers

- 10 Oligonucleotides have been designed thanks to the public library (NCBI) that contains the sequences of the contigs of genomic DNA and the assembled cDNAs of *Apis mellifera*. Lyophilized oligonucleotides (Eurofins MWG Operon) have been resuspended at 100 μ M in distilled water, aliquoted at 10 μ M and stored at -20°C until use.

SEQ ID NO:	Name	Séquence (5' to 3')
55	amric3-008S	CATGGCTGAAATAACAGATTTCG
56	amric3-005AS	CATGTGGAGGTGTACGTCGTTCTTG
59	amunc50-001S	CATGAAATATTCTACATCACCACCAGTAAG
60	amunc50-002AS	TATTCAAACAACCTCGATAATGATAAAATTCC
61	amunc74-001S	CATGGTAATAATAACGAAATTGATGTTTATTG
62	amunc74-002AS	CAATTATTCTAATCTTTCTTCATATGATTTG
57	amemc6-001S	CATGTTGGGAAAGATTAAAACAAAACAAG
58	amemc6-002AS	CTTTTAATCAGTATACATGTACCATTCCATAT
31	amnachra1-001S	CATGGCGACGGCCATTTCTGTC
32	amnachra1-002AS	CTAGTCCTCCTCGGGACCCATG
33	amnachra2-001S	CATGATACTCCAGACGATCATCTTGATCC
34	amnachra2-002AS	CTACACGAGAGTTTCCAAAAAGTCATCG

35	amnachra3-001S	CATGATGAAGAGCCTGGTGGGGATC
36	amnachra3-002AS	TTAGAGGCTCGTAACGATGTGTGG
37	amnachra4-001S	CATGCCCCCATAATAGGGGAAAC
38	amnachra4-002AS	CTATTGTGGCGGACAGTTTACCAC
39	amnachra5-003S	CATGTCGCCTTTGGTCCTGTTC
40	amnachra5-002AS	CTCTTAACCCTCTTTGGCAATGTTCG
41	amnachra6-001S	CATGCGCGCAAGTAGTGTATTACAAGC
42	amnachra6-002AS	CTTATTGGACGATTATGTGTGGCGC
43	amnachra7-001S	CATGAGACGTTGGACTCTCATGGCG
44	amnachra7-002AS	GAATCACGTGACGATGATGTGTGGC
45	amnachra8-001S	CATGTTTAAAATGCAAATATTGACGCTTGG
46	amnachra8-002AS	TTATCCTTCTGGAGAAATGTCTATATTTGG
47	amnachra9-001S	CATGAAAATGAGAATAATAACAGCTCTTGG
48	amnachra9-002AS	TCACGTGGATGGTACAAGAGTGATC
49	amnachrb1-001S	CATGCATAATATTTGCTCGAGGCTCG
50	amnachrb1-002AS	TTATTTTCCACGGTAGATCTCTATTATATG
51	amnachrb2-001S	CATGTAAACATGAAGAATATATTCCTCCG
52	amnachrb2-007AS	GAAAAAGATTAACACAAGTTACATTCCATAAG TTAAC
53	ceric3-001S	CATGCCAAAACTGAACGGCGTC
54	ceric3-002AS	TCAAGTCTTTTTAGGTCTCCGCCTTC
63	amric3-007AS	CAAGGCTCCACAGATTCAGATTTGATTC

Table 1. List of primers used to identify nAChR-gated ion channel subunits and their chaperones.

Electrophysiology.

RNA preparation

Plasmids coding for Am-nAChR α and β subunits.

- 5 The nAChR α and β subunits of the nAChR gated ion channel, as obtained above, have been linearized at a restriction site localized in the 3' sequence just following the STOP codon using the appropriate restriction enzyme (Table 2). The reaction medium (volume of 50 μ l) contained 10 μ g of plasmid, 3 μ l of the restriction enzyme (New England Biolabs France, Evry, France), 5 μ l of 10X reaction buffer provided by the
10 manufacturer and H₂O to 50 μ l. The reaction was then incubated for 3 hours at 37°C. The linearized plasmids were then purified using the NucleoSpin Extract II (Macherey-Nagel EURL, Hoerd, France) kit following manufacturer instructions, and resuspended at a concentration of 1 μ g/ μ l using deionized water (concentration was verified by measuring the OD at 260 nm with a Biophotometer (Eppendorf France SAS, Le Pecq,
15 France)). The efficiency of the linearization was also checked by agarose gel using 3 μ g of each plasmid.

- RNA for each subunit was then obtained by *in vitro* transcription using the kit T3- or T7- mMessage mMachine (Life Technologies, Saint Aubin, France); following manufacturer recommendations (3-4 hours at 37°C). mRNA were then purified by using
20 the RNeasy Mini Kit (Qiagen SAS, Courtaboeuf, France), and resuspended in desioned water at a concentration of 1 μ g/ μ l (verified by measuring the OD at 260 nm with a Biophotometer (Eppendorf France SAS, Le Pecq, France). The length of each mRNA was also verified on agarose gel using 0.5 μ g of RNA. Each mRNA was then aliquoted at 2 μ l and stored at -20°C for further use.

cDNA SEQ ID	Vector	Enzyme	Polymerase
Am-nAChR α 1 SEQ ID NO: 1	pCMV-PL10	NotI	T7
Am-nAChR α 2 SEQ ID NO: 2	pCMV-PL10	NotI	T7
Am-nAChR α 3 SEQ ID NO: 3	pCMV-PL10	NotI	T7
Am-nAChR α 4 SEQ ID NO: 4	pCMV-PL10	NotI	T7
Am-nAChR α 5 SEQ ID NO: 5	pCMV-PL10	NotI	T7
Am-nAChR α 6 SEQ ID NO: 6	pCMV-PL10	NotI	T7
Am-nAChR α 7 SEQ ID NO: 7	pCMV-PL10	NotI	T7
Am-nAChR α 8 SEQ ID NO: 8	pCMV-PL10	NotI	T7
Am-nAChR α 9 SEQ ID NO: 9	pCMV-PL10	NotI	T7
Am-nAChR β 1 SEQ ID NO: 10	pCMV-PL10	NotI	T7
Am-nAChR β 2 SEQ ID NO: 11	pCMV-PL10	NotI	T7
Am-ric3a SEQ ID NO: 12	pCMV-PL10	NotI	T7
Am-unc50 SEQ ID NO: 14	pCMV-PL10	NotI	T7
Am-unc74 SEQ ID NO: 15	pCMV-PL10	NotI	T7
Am-emc6 SEQ ID NO: 13	pCMV-PL10	NotI	T7
Ce-ric3 SEQ ID NO: 64	pCMV-PL10	NotI	T7

Table 2. cDNA, vector, restriction enzyme and RNA polymerases used for in vitro transcription.

Oocyte preparation

The *Xenopus laevis* female (from CRBM, UMR 5237, CNRS, Montpellier) was first anaesthetized by immersion using MS222 (ethyl-aminobenzoate methane sulfonate, ref A5040, Sigma) at 0.2% (pH=7) diluted in water during 20-30 minutes. Then a small 1-2 cm incision on the abdomen in between the midline and the lateral aspect of the

abdomen was made through the fascia and muscle to visualize the oocytes. Fascia and muscle were picked up with forceps before cutting to avoid cutting the liver. Oocyte strands were then gently externalized cut and placed in a Petri dish filled with the OR2 solution. The incision was closed by suturing both the fascia and skin layer in two layers using surgical thread. The *Xenopus* was then allowed to recover in dedicated tank water for 1-2 hours.

Oocytes were then washed 3 times with OR2 and transferred in a 50 ml Falcon tube filled 30 ml of OR2 supplemented with collagenase 1A (1 mg/ml ; ref C9891 Sigma), and placed in an orbital shaker for 2-3 hours. The proper enzymatic digestion of the follicular cell layer was followed by inspection of the oocytes under a 30X binocular. After completion oocytes were washed 2-3 times with OR2 and 2 times with ND96S (Table 3). Nicely isolated stage VI oocytes were selected and collected in batches of 30 oocytes in 30mm Petri dishes for injection.

OR2			ND96S		
	mM	g/l		mM	g/l
NaCl	82.5	4.81	NaCl	96	5.6
KCl	2	0.15	KCl	2	0.15
MgCl ₂	1	0.2	CaCl ₂	1.8	0.264
Hepes	5	1.19	MgCl ₂	1	0.2
			HEPES	5	1.19
pH=7.2 (NaOH)			Pyruvate	2.5	0.275
			Gentamicyne	0.05	1ml
			pH=7.5-7.6 (NaOH)		

Table 3. OR2 and ND96S solution.

15 *RNA or cDNA injection*

RNA injection was performed using glass pipets (Clark Electromedical Instrument CG150T1) pulled using a Sutter Inst. P30 microelectrode puller, giving a final sharp tip a 2-5 μ m. Under a binocular microscope, the pipette was then mounted on a micromanipulator and connected a homemade pressure-injection system. The pipette is

first filled with the RNA mixture by backfilling the tip. The Tip is immersed in a drop (2 μ L) of the RNA mixture, and filled by connecting the pipet, via the injection system, to vacuum. The 1 μ l of RNA are taken by the pipet with special care to avoid any air bubble. Then a batch of 20-30 oocytes are injected individually, under visual inspection with the microscope, with 25-50 nl of RNA mixture using the injection apparatus. The pipette is changed for each RNA mixture. For RNA, the point of injection on the oocytes is the equatorial ring, for DNA injection, the point of injection is the middle of the black/brown animal pole.

The mixture of RNA that have been used here are:

- 10 - Am-nAChR α x subunit alone or in combination with another Am-nAChR α x and/or Am-nAChR β y and/or Am-Ric3, and/or [Am-unc50 + Am-unc74 + Am-emc6],

With $x \in [1..9]$ and $y \in [1,2]$.

The final concentration of RNA was always 1 μ g/ μ l.

- 15 After injection, each batch of oocytes was placed again on the orbital shaker (1 rotation /2sec) for 2-5 days prior recording, with the incubation medium (ND96S) renewed daily.

Electrophysiological measurements

Two electrodes voltage-clamp set-up

- 20 A home-made recording chamber (50 μ l) is placed under a stereomicroscope and connected to an array of 8 reservoirs (50 ml syringes) containing the various solutions. The flow of solution from each syringe can individually be automatically switched ON or OFF by micro-electrovalves connected to the voltage-clamp recording software version 9.0 of the pClamp program (Axon Inst., Molecular devices).

- 25 The chamber is electrically connected to the ground by Agar bridges. Clark capillaries (with filament, GC150F10) are bent at $\sim 120^\circ\text{C}$ under flame and subsequently immersed in 60mm Petri dishes filled with almost boiling agar (high gel strength) dissolved at 1%

in 3M KCl (5-10 capillaries can be placed per dish). When immersed, these bridges are usually filled naturally (by capillarity) by the hot agar solution. After cooling, two Agar-bridges are “dissected” from the agar and placed in the bath-electrode holder previously filled with 3M KCl. For two-electrodes voltage-clamp, voltage and current electrodes
5 were pulled from Clark Electromedical Instrument borosilicate glass capillaries (GC150T-10) using a P-97 Sutter Instrument Company puller. They have a resistance of 0.5-2 M Ω when filled with 3M KCl.

In both two-electrodes voltage-clamp and single-channel recordings voltage protocols and current recordings are made using version 9.0 of the pClamp program (Axon Inst.,
10 Molecular devices) running on a PC computer connected, via Digidata 1200 interface to the Geneclamp 500 amplifier (Axon Instruments, Inc.) for two-electrodes voltage-clamp or to the Axopatch 200B amplifier (Axon Instruments, Inc.) for single channel recordings. Thus, voltage-command, sampling, acquisition and analysis are done using pClamp program. Additional analysis was performed using the Microsoft Excel
15 software. All experiments are performed at room temperature (20-25°C).

Typically, an oocyte, injected 2 to 5 days before with a given mixture of RNA is placed in the recording chamber filled with the desired solution (usually Na100). Junction potentials (typically less than 3-5 mV) at the two electrodes are first cancelled using the zero button of the amplifier in Na100 solution, and the electrodes resistance is checked
20 before introduction into the oocyte (between 0.3 to 1.5 M Ω). Both electrodes are then impaled into the oocyte and the resting membrane potential is measured (typically -30/-50 mV). The oocyte is then voltage-clamped usually at – 60 mV. The effect of Ach on expressed channels is then check by switching the perfusion system from on reservoir of control solution (i.e. ND96 or Na100) from one containing ACh at a given
25 concentration (usually between 100 to 1000 μ M), or a neonicotinoid (imidacloprid, clothanidin or thiamethoxam). Sometime, the effect of a test compound can be determined directly, by applying 50 μ l of the compound directly to the bath (with the main perfusion stopped) at the final working concentration (usually 100-1000 μ M), after proper dilution from a stock solution (usually 10 mM in water or DMSO or

ethanol) into the desired recording solution. We assume that there is no dilution in the recording chamber which has a volume of around 50 μ l.

Membrane currents are measured either at steady voltage as the difference between the current amplitudes before and during the application of ACh. These measures can also
5 be done at different membrane potential (steady potential from -60 to -100 mV or voltage –ramps from -80 mV to +20 mV, with the change in membrane conductance followed by the modification in the slope of the current-voltage curve: current recorded during the voltage ramp, with the corresponding voltage axis). In these conditions, washing-out of the test compound is done by switching-on the gravity-driven perfusion
10 of the chamber with the same solution without test compound. Similar experiments are performed with oocytes injected with different nAChR subunits and chaperone proteins RNA.

The tested compound can either decrease or increase ACh-induced current or modified other parameters, such as the channel kinetics, or channel selectivity for example. Such
15 modifications can be considered as toxic for bees, thus revealing a potential toxicity of these products for bees.

Dose-response curves are obtained by measuring the ACh-induced current amplitude (I_{ACh}), as measured before, for different concentrations of ACh or the tested compounds. From these measured, the $I_{ACh} = f(\log([ACh]))$ is constructed and a non-linear regression
20 using the following equation is performed.

$$I_{ACh} = I_{AChMax} / (1 + \exp([ACh] - EC_{50})/h)$$

Where I_{ACh} is the ACh-induced current, I_{AChMAX} is the current amplitude recorded for a saturating ACh concentration, $[ACh]$ are the various ACh concentrations perfused, “h” is a slope factor. For each nAChR subunit combinations showing functional
25 expression the EC_{50} and the “h” factor is then calculated.

Ionic selectivity is calculated by measuring the current reversal potentials of ACh-induced currents in recording solutions. Current Reversal potential ($E_{rev}(X)$) is then measured as the potential at which the ACh-induced current is 0 during voltage ramps.

This measure is done after digital subtraction of traces recorded before and after ACh application. All these measurements are usually performed on 3-15 different oocytes of each batch for each concentration or compounds, and the resulting values represents the average result between these oocytes. The statistical significance is tested using the student t-test at 5%.

CLAIMS

1. A nucleic acid sequence of at least one subunit α of the nicotinic acetylcholine receptor of an arthropod as set forth in SEQ ID NO: 1 to 9 or a variant thereof.
- 5 2. A nucleic acid sequence of at least one subunit β of the nicotinic acetylcholine receptor of an arthropod as set forth in SEQ ID NO: 10 to 11 or a variant thereof.
3. A nucleic acid sequence of the chaperone protein of the nicotinic acetylcholine receptor of an arthropod as set forth in SEQ ID NO: 12 to 15 or a variant thereof.
- 10 4. A vector comprising at least one nucleic acid sequence according to any one of claims 1 to 3.
5. An isolated cell transfected with at least one vector of claim 4.
6. A functional nicotinic acetylcholine receptor (nAChR) channel comprising at least one subunit α of nAChR encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof according to claim 1.
- 15 7. A functional nAChR channel comprising at least one subunit α encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof according to claim 1 and at least one chaperone protein encoded by the nucleic acid sequence selected from the group comprising SEQ ID NO: 12 to 15 or a variant thereof according to claim 3.
- 20 8. A functional nicotinic acetylcholine receptor (nAChR) channel comprising at least one subunit α of nAChR encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof according to claim 1 and at least one subunit β encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 10 to 11 or a variant thereof according to claim 2.
- 25 9. A functional nAChR channel of an arthropod comprising at least one subunit α of nAChR encoded by a nucleic acid sequence selected from the group comprising SEQ ID NO: 1 to 9 or a variant thereof according claim 1, at least one subunit β of

nAChR encoded by the nucleic acid sequence selected from the group comprising SEQ ID NO: 10 to 11 or a variant thereof according to claim 2 and at least one chaperone protein encoded by nucleic acid sequences selected from the group comprising SEQ ID NO: 12 to 15 or a variant thereof according to claim 3.

- 5 **10.** A vector comprising the channel according to claim 6 or 7 or 8 or 9.
- 11.** An isolated cell transfected with at least one vector of claim 10, and expressing the channel according to claim 6 or 7 or 8 or 9, preferably said cell is an oocyte of *Xenopus*.
- 12.** An *in vitro* method to determine the effect of a test compound on the modulation
10 of activity of a nAChR channel of an arthropod comprising:
 a. contacting an isolated cell according to of claim 5 or 11 with at least one test compound,
 b. measuring the effect of said test compound on the channel activity, and comparing said effect to the effect without test compound or to the effect of a
15 reference value, thereby determining a modulation of activity of said channel.
- 13.** The method according to claim 12, for determining the toxicity of a test compound on an arthropod.
- 14.** An *in vitro* method for screening compounds that modulate the nAChR channel activity of an arthropod comprising:
20 a. contacting an isolated cell according to claim 5 or 11 with at least one test compound,
 b. measuring the effect of said compound on the nAChR channel activity, and
 comparing said effect to the effect without test compound or to the effect of a
25 reference value, thereby determining a modulation of activity of said nAChR channel.
- 15.** A kit comprising at least one vector according to claim 4 or 10, or an isolated cell according to claim 5 or 11 and reagents.

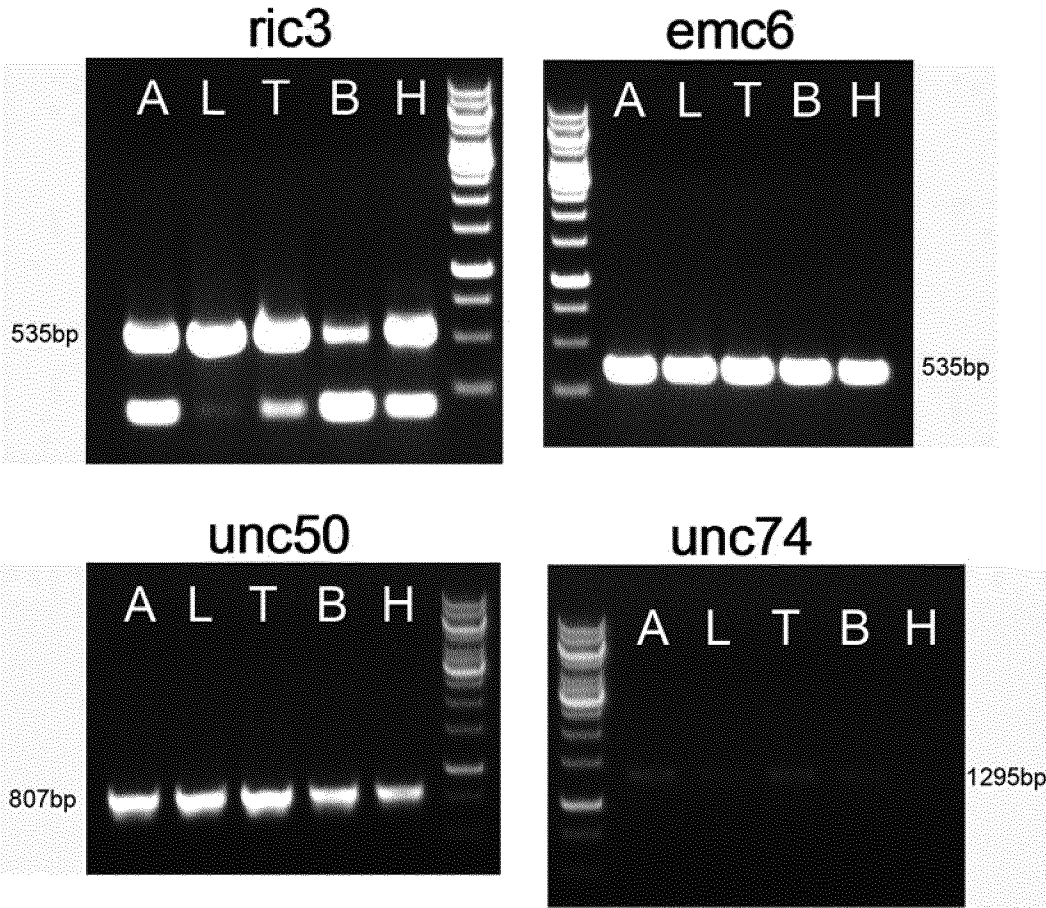


FIG. 1

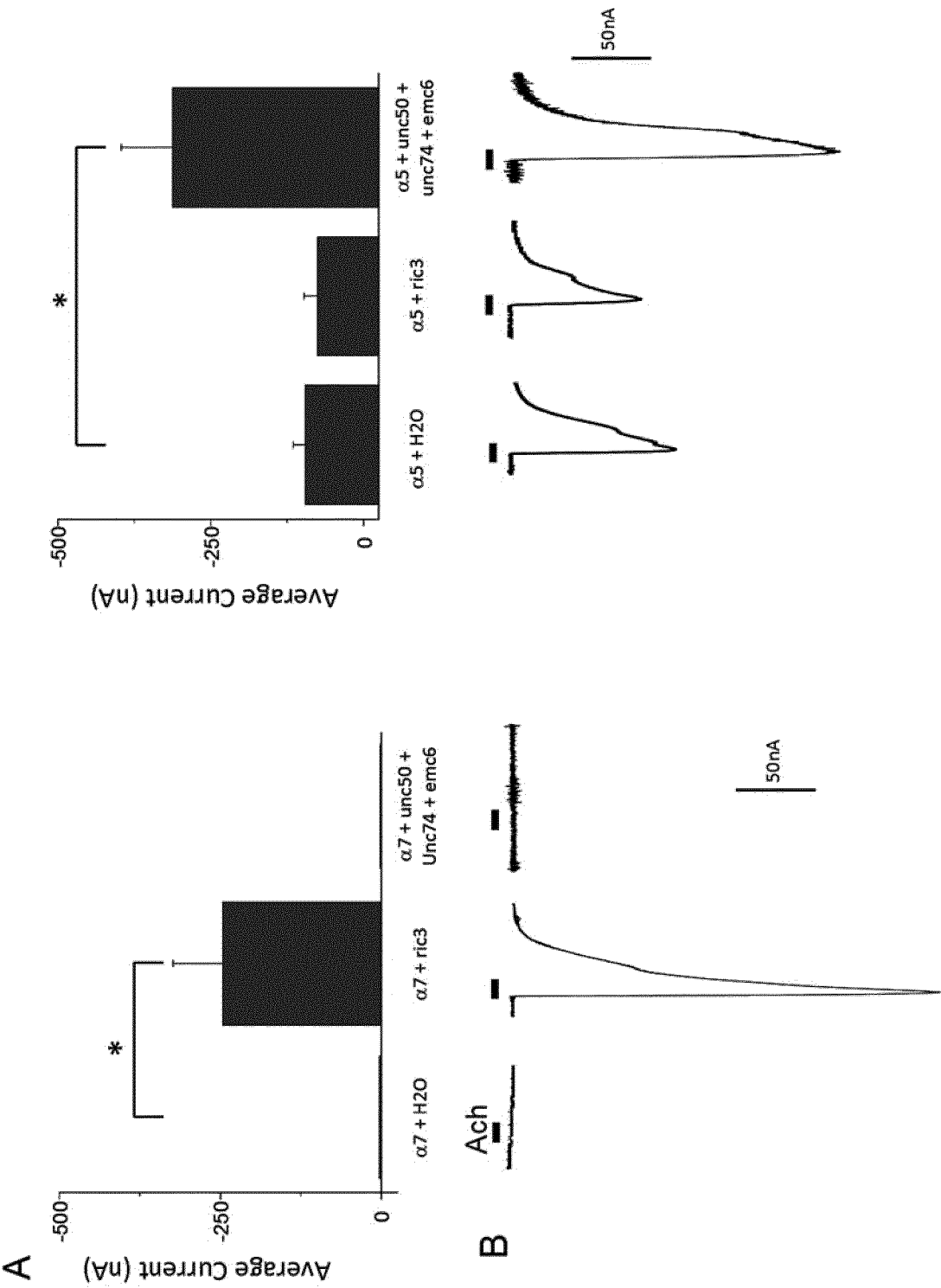


FIG. 2

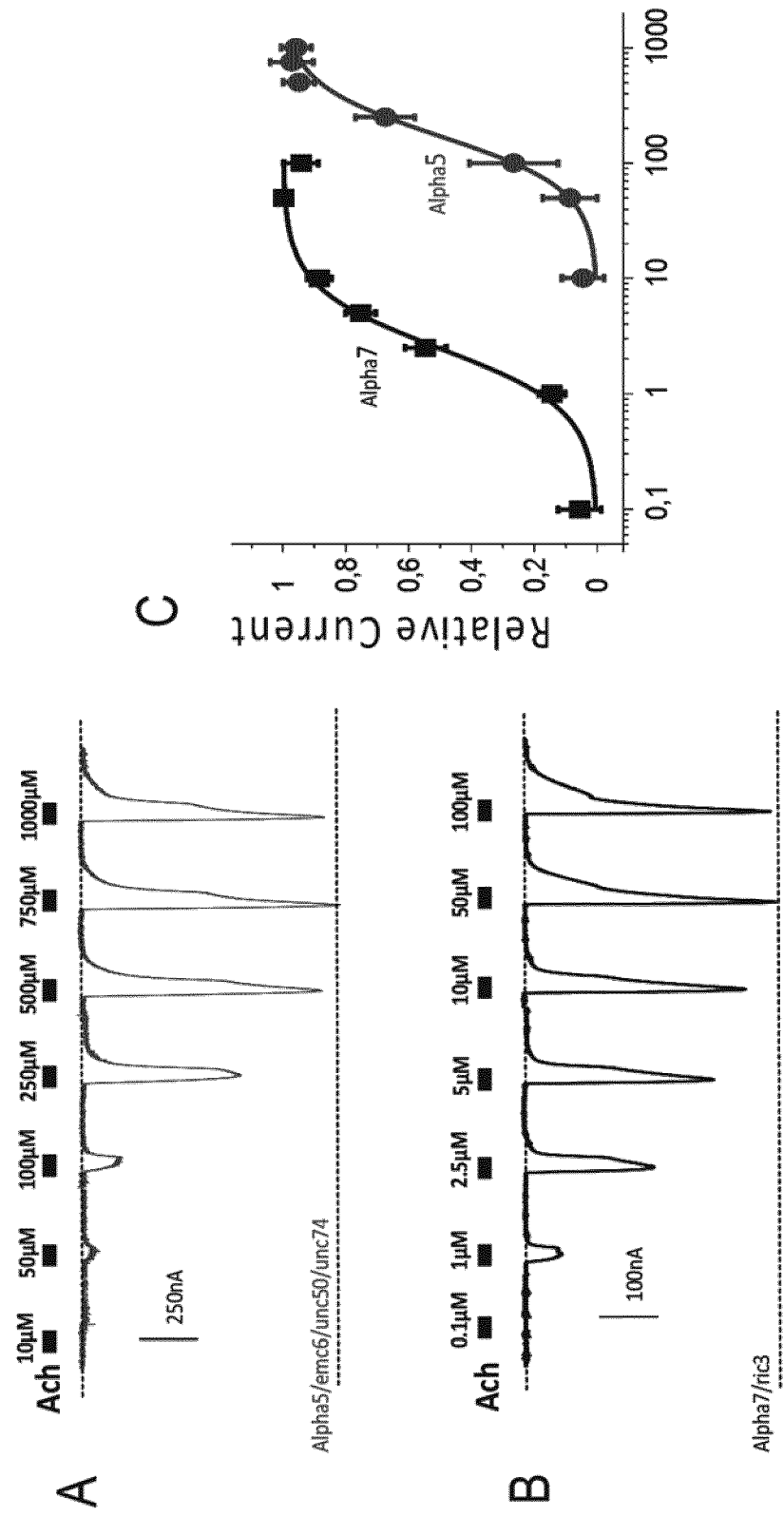


FIG. 3

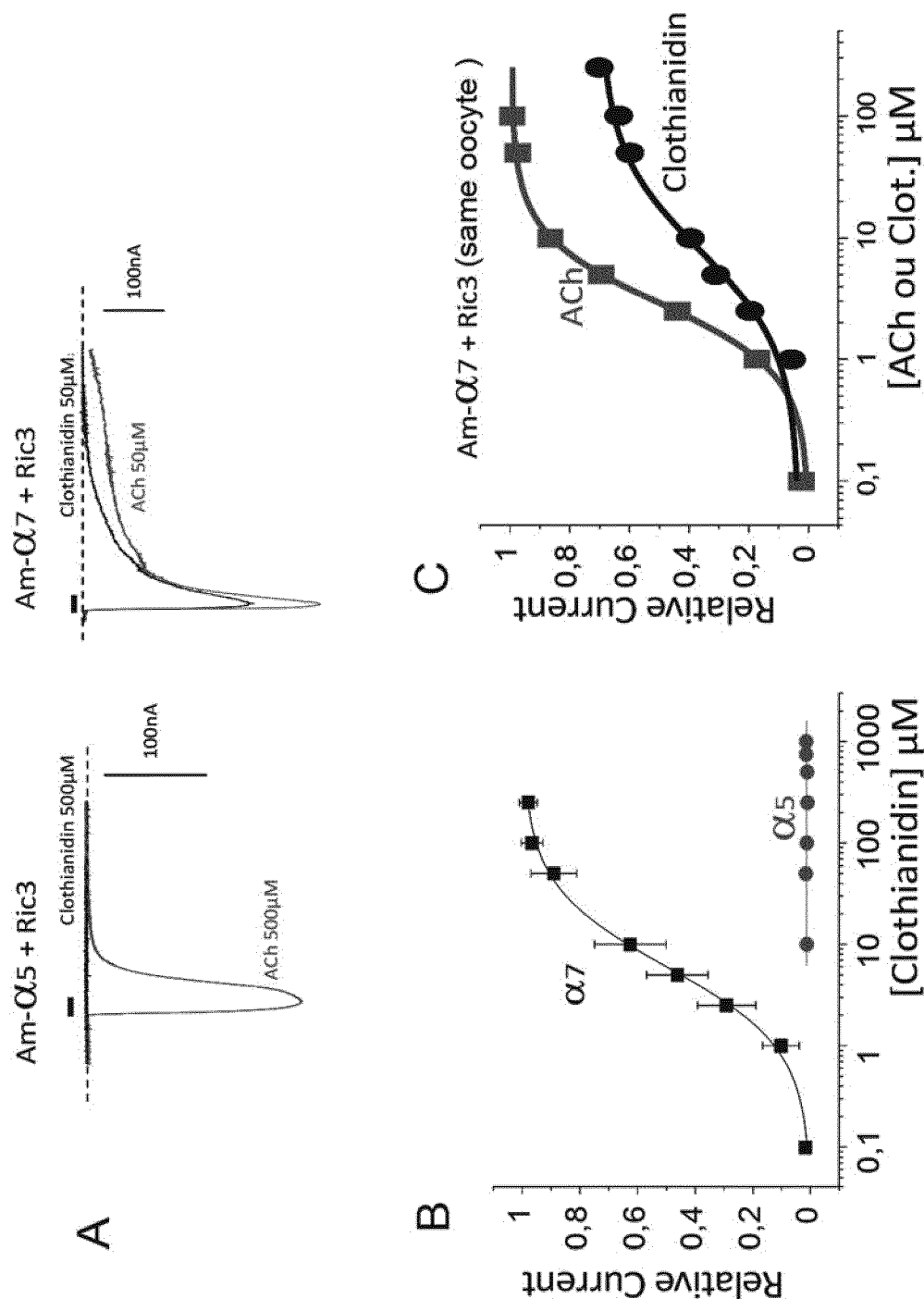


FIG. 4

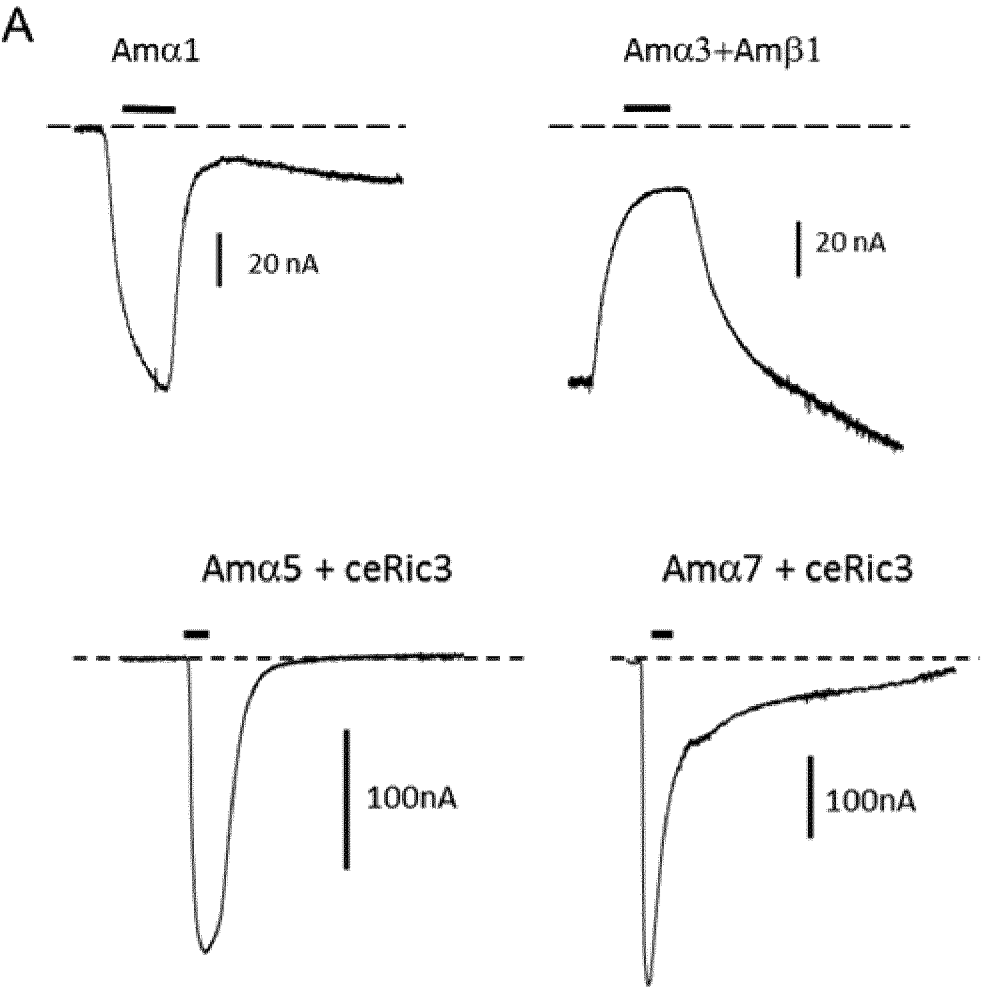


FIG. 5A

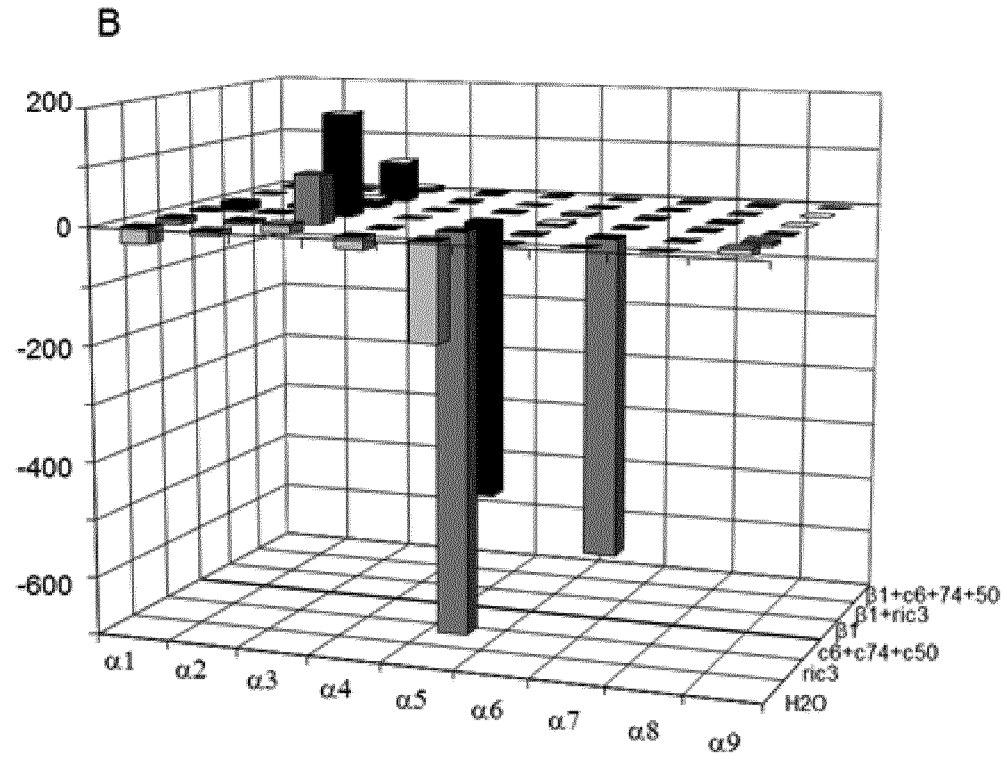


FIG. 5B

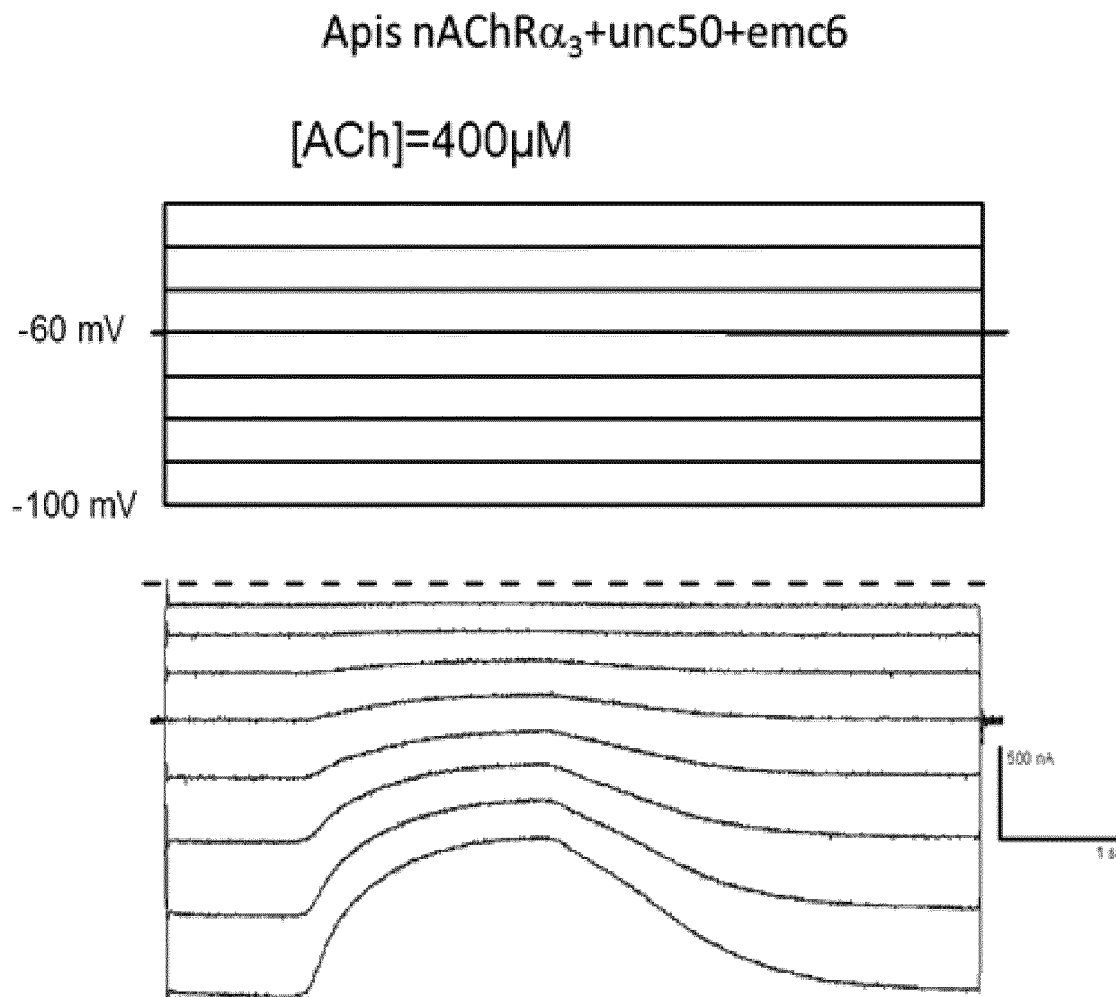


FIG. 6

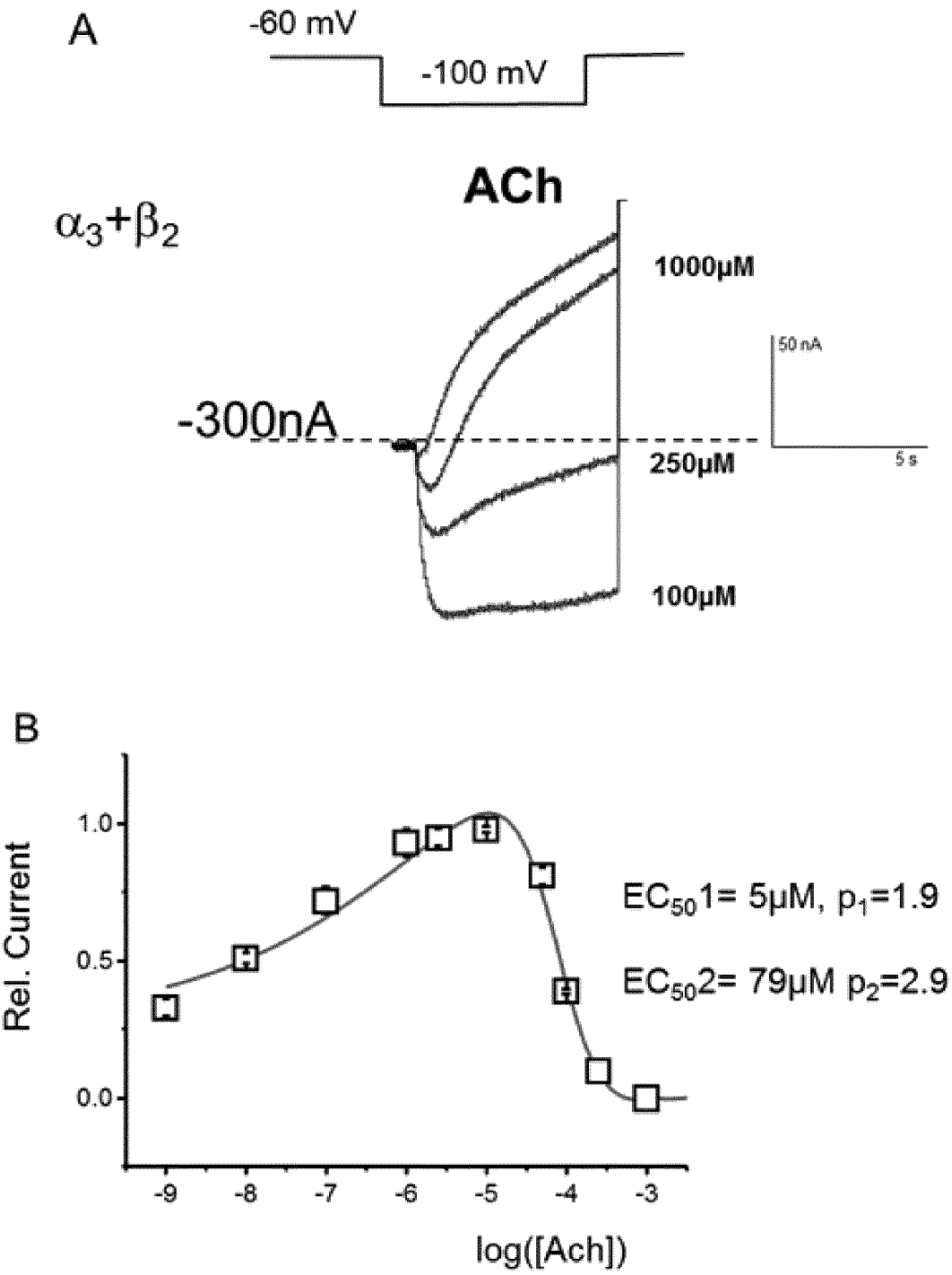


FIG. 7

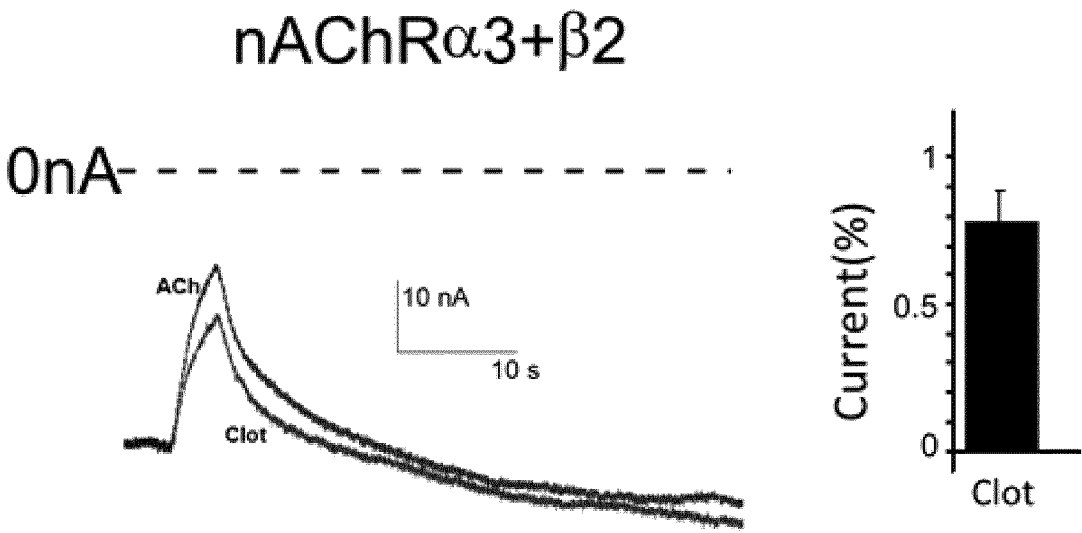


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066616

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/705 C07K14/47 G01N33/68
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHRISTINE G ELSIK ET AL: "Finding the missing honey bee genes: lessons learned from a genome upgrade", BMC GENOMICS, vol. 15, no. 1, 30 January 2014 (2014-01-30), page 86, XP021175420, BIOMED CENTRAL LTD, LONDON, UK ISSN: 1471-2164, DOI: 10.1186/1471-2164-15-86	1-5,10, 15
Y	abstract ----- -/--	6-9, 11-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 September 2016

Date of mailing of the international search report

12/10/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Griesinger, Irina

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066616

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. K. JONES ET AL: "The nicotinic acetylcholine receptor gene family of the honey bee, <i>Apis mellifera</i> ", GENOME RESEARCH, vol. 16, no. 11, 25 October 2006 (2006-10-25), pages 1422-1430, XP055223583, ISSN: 1088-9051, DOI: 10.1101/gr.4549206 table 1	1-15
X	----- DATABASE EMBL [Online] 2 July 2015 (2015-07-02), "Apis mellifera clone 1113L6.5 nicotinic acetylcholine receptor alpha1 subunit (nAChRalpha1) mRNA, complete cds.", XP002748811, retrieved from EBI accession no. EM STD:KJ939588 Database accession no. KJ939588	1,4,5, 10,15
Y	the whole document	2,3,6-9, 11-14
X	----- DATABASE EMBL [Online] 2 July 2015 (2015-07-02), "Apis mellifera clone 1013L21.1 nicotinic acetylcholine receptor alpha3 subunit (nAChRalpha3) mRNA, complete cds.", XP002748812, retrieved from EBI accession no. EM STD:KJ939590 Database accession no. KJ939590	1,4,5, 10,15
Y	the whole document	2,3,6-9, 11-14
X	----- DATABASE EMBL [Online] 2 July 2015 (2015-07-02), "Apis mellifera clone 1113L11.2 nicotinic acetylcholine receptor beta1 subunit (nAChRbeta1) mRNA, complete cds.", XP002748813, retrieved from EBI accession no. EM STD:KJ939597 Database accession no. KJ939597	2,4,5, 10,15
Y	the whole document	1,3,6-9, 11-14
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066616

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] 2 July 2015 (2015-07-02), "Apis mellifera clone 1013L19.2 receptor associated protein RIC-3, alternatively spliced (Ric-3) mRNA, complete cds.", XP002748814, retrieved from EBI accession no. EM STD:KJ939599 Database accession no. KJ939599	3-5,10,15
Y	the whole document	1,2,6-9,11-14

X	DATABASE EMBL [Online] 2 July 2015 (2015-07-02), "Apis mellifera clone 1013L4.1 ER membrane protein complex subunit6-like protein (LOC551477) mRNA, complete cds.", XP002748815, retrieved from EBI accession no. EM STD:KJ939604 Database accession no. KJ939604	3-5,10,15
Y	the whole document	1,2,6-9,11-14

X	DATABASE EMBL [Online] 2 July 2015 (2015-07-02), "Apis mellifera clone 1013L5.1 unc-50-like protein (LOC550684) mRNA, complete cds.", XP002748816, retrieved from EBI accession no. EM STD:KJ939605 Database accession no. KJ939605	3-5,10,15
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X	BOULIN T ET AL: "Functional reconstitution of Haemonchus contortus acetylcholine receptors in Xenopus oocytes provides mechanistic insights into levamisole resistance", BRITISH JOURNAL OF PHARMACOLOGY, vol. 164, no. 5, November 2011 (2011-11), pages 1421-1432, XP002748817, abstract	1-15

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066616

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOULIN THOMAS ET AL: "Eight genes are required for functional reconstitution of the <i>Caenorhabditis elegans</i> levamisole-sensitive acetylcholine receptor (+ Supporting Information)", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 105, no. 47, November 2008 (2008-11), pages 18590-18595+2, XP002748818, ISSN: 0027-8424, DOI: 10.1073/pnas.0806933105 abstract -----	1-15
X	WO 2006/119497 A2 (UNIV UTAH RES FOUND [US]; WILLIAMS DANIEL C [US]; JORGENSEN ERIK M [US]) 9 November 2006 (2006-11-09) claims 6,8,16,28 -----	1-15
X	MILLAR N S: "RIC-3: a nicotinic acetylcholine receptor chaperone", BRITISH JOURNAL OF PHARMACOLOGY, vol. 153, no. Suppl. 1, March 2008 (2008-03), pages S177-S183, XP002748819, ISSN: 0007-1188 abstract -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/066616

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006119497 A2	09-11-2006	US 2010160217 A1 WO 2006119497 A2	24-06-2010 09-11-2006
