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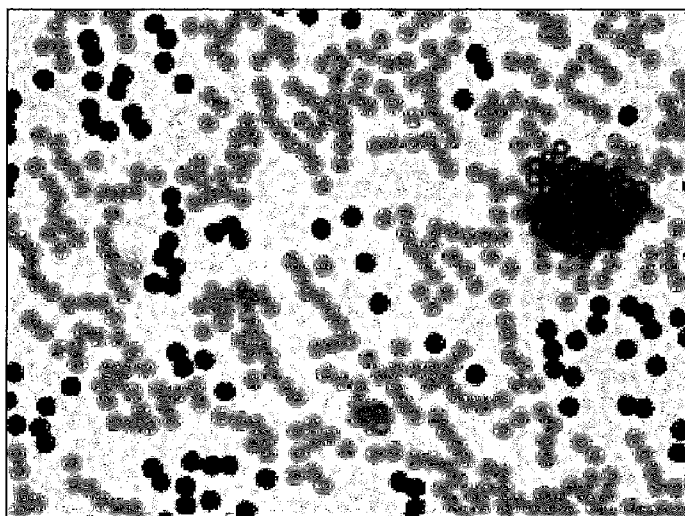
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[Continued on next page]

(54) Title: BOTULINUM TOXIN SCREENING ASSAYS

FIG. 5.



(57) Abstract: Methods for detecting BoNT/A activity in a sample, methods for screening molecules able to compete with BoNT/A receptor binding, methods for reducing BoNT/A activity in a human and methods of marketing a neurotoxin capable of selectively binding to a FGFR2, a FGFR3, a SV2, or any combination thereof, to a governmental or regional regulatory authority.



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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
 - *with international search report (Art. 21(3))*
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Botulinum Toxin Screening Assays

[01] This is an international patent application that claims priority to U.S. Patent Application Serial No. 12/469,569, filed May 20, 2009, a continuation in part application that claims priority under 35 U.S.C. § 120 to U.S. Patent Application Serial No. 12/047,441, filed March 13, 2008, a continuation in part application that claims priority under 35 U.S.C. § 120 to U.S. Patent Application Serial No. 10/598,073, filed August 17, 2006, a national stage application under 35 U.S.C. § 371 of International Application PCT/US2005/006421, filed on February 23, 2005, which claims the benefit of U.S. Provisional Application Serial No. 60/547,591, filed February 24, 2004, each of which is hereby incorporated by reference in its entirety.

[02] The myorelaxant properties of Botulinum toxins (BoNTs) are being exploited in a wide variety of therapeutic and cosmetic applications, see e.g., William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). For example, BoNTs therapies are proposed for treating dystonia, see e.g., Kei Roger Aoki, et al., *Method for treating Dystonia with Botulinum Toxin C to G*, U.S. Patent No. 6,319,505 (Nov. 20, 2001); pain, see e.g., Kei Roger Aoki, et al., *Method for Treating Pain by Peripheral Administration of a Neurotoxin*, U.S. Patent No. 6,464,986 (Oct. 15, 2002); muscle injuries, see e.g., Gregory F. Brooks, *Methods for Treating Muscle Injuries*, U.S. Patent No. 6,423,319 (Jul. 23, 2002); cardiovascular diseases, see e.g., Gregory F. Brooks, *Methods for Treating Cardiovascular Diseases with Botulinum Toxins*, U.S. Patent Publication No. 2003/0185860 (Oct. 2, 2003); neuropsychiatric disorders, see e.g., Steven Donovan, *Therapeutic Treatments for Neuropsychiatric Disorders*, U.S. Patent Publication No. 2003/0211121 (Nov. 13, 2003); lower back pain, see e.g., Kei Roger Aoki, et al., *Botulinum Toxin Therapy for Lower Back Pain*, U.S. Patent Publication No. 2004/0037852 (Feb. 26, 2004); as well as other neuromuscular disorders, see e.g., Kei Roger Aoki, et al., *Multiple Botulinum Toxins for Treating Neuromuscular Disorders and Conditions*, U.S. Patent Publication No. 2001/0021695 (Sep. 13, 2001); Kei Roger Aoki, et al., *Treatment of Neuromuscular Disorders and Conditions with Different Botulinum*, U.S. Patent Publication No. 2002/0010138 (Jan. 24, 2002); Kei Roger Aoki, et al., *Use of Botulinum Toxins for Treating Various Disorders and Conditions and Associated Pain*, U.S. Patent Publication No. 2004/0013692 (Jan. 22, 2004). Additional proposed uses of BoNTs as biopharmaceutical neuromodulators has expanded to cover a wide variety of treatments targeting certain disorders that lack a neuromuscular basis. For example, the effects on the autonomic nervous system has allowed the development of a Botulinum toxin serotype A (BoNT/A) therapy for treating axillary hyperhidrosis or sweating, and reports indicate BoNT/A may be an effective treatment for myofascial pain and tension, stroke, traumatic brain injury, cerebral palsy, gastrointestinal motility disorders, urinary incontinence cancer and migraine headaches. Lastly, cosmetic and other therapeutic applications are widely known. In fact, the expected use of BoNTs in both therapeutic and cosmetic treatments of humans is anticipated to expand to an ever widening range of diseases and ailments that can benefit from the myorelaxant properties of these toxins.

[03] The growing clinical and therapeutic use of botulinum toxins necessitates the pharmaceutical industry to use accurate assays for BoNT activity in order to, *e.g.*, ensure accurate pharmaceutical formulations and monitor established quality control standards. In addition, given the potential danger associated with small quantities of BoNT in foodstuffs, the food industry requires BoNT activity assays, for example, to validate new food packaging methods and to ensure food safety. Additionally, BoNT activity assays are useful in identifying modulators of BoNT activity, for example, modulators that reduce BoNT activity which can be useful as a toxin antidote and modulators that increase BoNT activity which can be useful in creating more potent or longer lasting pharmaceutical formulations. The present specification provides novel BoNT assays for detecting the presence or activity of a BoNT useful for various industries, such as, *e.g.*, the pharmaceutical and food industries, and provides related advantages as well.

BRIEF DESCRIPTION OF THE DRAWINGS

[04] **FIG. 1** shows a schematic of the current paradigm of the BoNT/A intoxication mechanism. This intoxication process can be described as comprising four steps: 1) receptor binding, where BoNT/A binds to a BoNT/A receptor system initiates the intoxication process; 2) complex internalization, where after BoNT/A binding, a vesicle containing a toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events occur, including changes in the internal pH of the vesicle, formation of a channel pore comprising the translocation domain of a BoNT/A heavy chain, separation of the BoNT/A light chain from the heavy chain, increased enzymatic activity of the light chain; and release of the light chain into the cytoplasm and 4) enzymatic target modification, where the light chain of BoNT/A proteolytically cleaves its target SNARE substrates, such as, *e.g.*, SNAP-25.

[05] **FIG. 2** shows a schematic of an FGFR3 and the alternatively spliced exons that result in FGFR3IIb and FGFR3IIc. The top diagram shows a generalized drawing of a FGFR3. The extracellular domain comprises a signal peptide (box labeled SP), three Ig-like domains (loops labeled Igl, IgII and IgIII) and an acid box (box labeled acid). A single membrane spanning region comprises the transmembrane domain (box labeled TM). The cytoplasmic portion of the receptor comprises the tyrosine kinase domain. The middle diagram shows a generalized drawing of the exons encoding a FGFR3IIb isoform, where exon 9 is spliced out from the primary transcript during processing. The lower diagram shows a generalized drawing of the exons encoding a FGFR3IIc isoform, where exon 8 is spliced out from the primary transcript during processing.

[06] **FIG. 3** shows the results of electroporation of PURE-A into HIT-T15 cells. FIG. 3a shows the results of an inhibition of insulin release assay. The graph indicates that the addition of glucose to 25 mM induced insulin secretion from untreated cells (control) and cells subjected to electroporation without the addition of PURE-A (Electroporation No PURE-A). However, HIT-T15 cells into which PURE-A was introduced (Electroporation No PURE-A) showed a decrease in insulin secretion from indicating these cells were unresponsive to induction of insulin secretion. FIG. 3b shows the results of a SNAP-25

cleavage assay. Western blot analysis identified the presence of a BoNT/A SNAP-25₁₉₇ cleavage product in PURE-A treated cells (Electroporation PURE-A), but not in either control (Control and Electroporation No PURE-A), with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product.

[07] **FIG. 4** shows the affects of electroporation of HIT-T15 cells over time. FIG. 4a shows the results on an inhibition release for insulin assay demonstrating that the presence of the toxin delayed growth in HIT-T15 cells when compared to controls, but toxin-treated cells were able to replicate normally after a recovery period. FIG. 4b shows a western blot analysis demonstrating that cleavage of SNAP-25 was detected at all time points tested when PURE-A was introduced into the cells, with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product.

[08] **FIG. 5** shows HIT-T15 cells, transformed with a human brain cDNA library and selected using magnetic beads to which BONT/A had been bound. Individual colonies are visible in the dish and are surrounded by magnetic beads.

[09] **FIG. 6** shows the results of an assay of insulin release from HIT-T15 cells containing the putative BONT/A receptor. Cells were exposed to 1 nM PURE-A and assayed for inhibition of insulin release upon glucose stimulation.

[010] **FIG. 7** shows the analysis of two isolated HIT-T15 cell isolates C6 and C7. FIG. 7a shows the reduction of insulin release in representative HIT-T15 transformants C6 and C7 upon incubation with BONT/A. FIG. 7b shows a western blot analysis demonstrating that cleavage of SNAP-25 was detected in clones C6 and C7 incubated with BONT/A, with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product.

[011] **FIG. 8** shows Western blot analysis identifying cells with high affinity uptake for a Clostridial toxin. FIG. 8a shows a Western blot analysis used to identify cells capable of BoNT/A uptake. The blot shows five cell lines treated with 1 nM of PURE-A overnight, with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product. FIG. 8b shows Western blot analysis used to evaluate the time necessary for BoNT/A uptake. The blots show either Neuro-2A cells or SH-SY5Y cells treated with 1 nM of PURE-A for various lengths of time, with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product. FIG. 8c shows a Western blot analysis used to evaluate the concentration range necessary of BoNT/A uptake. The blots show Neuro-2A cells treated with a range of PURE-A concentrations overnight, with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product.

[012] **FIG. 9** shows Western blot analysis evaluating the effects of ganglioside treatments used to increase uptake of a botulinum toxin. FIG. 9a shows a Western blot analysis evaluating the effects of ganglioside treatment on the uptake of BoNT/A. The blot shows Neuro-2A cells treated without or with 25 µg/mL of GT1b (- or +) and exposed overnight to three different concentrations of BoNT/A (12.5 pM, 25 pM or 50 pM), with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product. FIG. 9b shows a Western blot analysis evaluating the effects of ganglioside treatment on the uptake of BoNT/E. The blot shows Neuro-2A cells treated with either 25 µg/mL of GT1b, GQ1b, GD1a, GD1b or GD3 and exposed for approximately 5 hours to 14 nM of BoNT/E di-chain, with equal amounts of protein loaded per lane and probed with an antibody (SMI-81; Sternberger Monoclonals, Lutherville, MD) that detects the uncleaved SNAP-25₂₀₆ substrate and the BoNT/E SNAP-25₁₈₀ cleavage product.

[013] **FIG. 10** shows the results of a crosslinking experiment in Neuro-2A cells using a BoNT/A-SBED toxin. FIG. 10a shows the isolation of a complex of approximately 250 kDa from Neuro-2A cells containing the 150 kDa neurotoxin cross-linked to the putative BONT/A receptor. Bands were visualized with silver staining. FIG. 10b shows a Western blot analysis used to identify a BoNT/A receptor. The blots shows the presence of a single band corresponding to the 97 kDa FGFR3 (first panel) and two bands corresponding to the 150 kDa BoNT/A holotoxin and the 100 kDa BoNT/A heavy chain (second panel), with equal amounts of protein loaded per lane and probed with an antibody that detects either FGFR3 or BoNT/A.

[014] **FIG. 11** shows a Western blot analysis used to determine the presence of FGFRs in five different cell lines. Only antibodies selectively binding to FGFR3 detected bands that correlated with cell lines that contained a BoNT/A receptor.

[015] **FIG. 12** shows the results of a receptor competition experiment in Neuro-2a cells using BoNT/A and FGF ligands. FIG 12a shows a western blot analysis shows that both FGF1 and FGF2 effectively competed with BoNT/A for binding to the BoNT/A receptor, with equal amounts of protein loaded per lane and probed with antibody (SMI-81; Sternberger Monoclonals, Lutherville, MD) that detects the uncleaved SNAP-25₂₀₆ substrate and the BoNT/E SNAP-25₁₈₀ cleavage product. The appearance of the uncleaved SNAP-25₂₀₆ substrate was detected when as little as 1nM of FGF ligand was present and clearly visible when 5 nM of FGF ligands were present. Detectable levels of the BoNT/A SNAP-25₁₉₇ cleavage product was absent in FGF ligand treatments of 200 nM. FIG 12b shows a graphical representation of Western blot data from another receptor competition experiment indicating that FGF2 and FGF9, but not FGF10, effectively competed with BoNT/A for binding to the BoNT/A receptor.

[016] **FIG. 13** shows the results FGFR3 phosphorylation studies in Neuro-2A cells. FIG. 13 a shows a Western blot analysis indicating the presence of phosphorylated FGFR3 after exposure to FGF2 or BoNT/A. The blot shows Neuro-2A cells treated with either 5 nM FGF2 or 5 nM BoNT/A for various lengths of time, with equal amounts of protein loaded per lane and probed with an antibody that detects

FGFR3. FIG. 13b shows a Western blot analysis indicating the reduction of phosphorylated FGFR3 when exposed to increasing amounts of DMBI. The blot shows Neuro-2A cells treated with 5 nM FGF2 for 10 minutes, with equal amounts of protein loaded per lane and probed with an antibody that detects phosphorylated FGFR3. FIG. 13c shows a Western blot analysis indicating the reduction of SNAP-25₁₉₇ cleavage product when exposed to increasing amounts of DMBI. The blots show either Neuro-2A cells treated with 5 nM of BoNT/A for 10 minutes, with equal amounts of protein loaded per lane and probed with an antibody that detects the BoNT/A SNAP-25₁₉₇ cleavage product.

DETAILED DESCRIPTION

[017] The present invention is based on the identification of a cell surface receptors to which BoNT/A selectively binds as the first step to the selective intoxication of a neuron. The present specification, in part, discloses that the Fibroblast Growth Factor Receptor 3 (FGFR3) is useful as a BoNT receptor, such as, *e.g.*, a BoNT/A receptor. In addition, the present specification, in part, discloses that the Fibroblast Growth Factor Receptor 2 (FGFR2) is useful as a BoNT receptor, such as, *e.g.*, a BoNT/A receptor. In addition, the present disclosure identifies SV2 as a co-receptor of FGFR3 or FGFR2 which enhances binding of a BoNT to a BoNT receptor, such as, *e.g.*, a FGFR2 or a FGFR3. Further, the present disclosure identifies specific gangliosides which facilitate binding of a BoNT to a BoNT receptor and the internalization of these toxins within a neural cell., such as, *e.g.*, an increased binding of BoNT/A for a BoNT/A receptor using a ganglioside like GT1b; and an increased binding of BoNT/E for a BoNT/E receptor using a ganglioside like GQ1b, GD1a, GD1b or GD3.

[018] The present invention provides novel assays for detecting the presence or absence of an active BoNT/A. The novel methods disclosed in the present specification reduce the need for animal-based toxicity studies, yet serve to analyze multiple toxin functions, namely, binding and cellular uptake of toxin, translocation into the cell cytosol, and protease activity. As discussed further below, the novel methods of the present disclosure can be used to analyze crude and bulk samples as well as highly purified di-chain toxins and formulated toxin products and further are amenable to automated high throughput assay formats.

[019] Aspects of the present invention provide methods of detecting BoNT/A activity by contacting a sample to a cell that contains a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2, a FGFR3 and a SV2, or a FGFR2, a FGFR3, and a SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. Other aspects of the present invention provide methods of detecting BoNT/A activity by contacting a sample to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3, and an exogenous SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell

relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. Yet other aspects of the present invention provide methods of detecting BoNT/A activity by contacting a sample to a cell that transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3, and an exogenous SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. Other aspects of the present invention provide methods of detecting BoNT/A activity by contacting a sample to a cell that stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3, and an exogenous SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[020] Other aspect of the present invention provide methods of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2 complex, a FGFR3 and a SV2 complex, or a FGFR2, a FGFR3 and a SV2 complex, wherein said selective binding reduces the ability of BoNT/A to bind to said FGFR3. Other aspect of the present invention provide methods of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2 complex, a FGFR3 and a SV2 complex, or a FGFR2, a FGFR3 and a SV2 complex, wherein said selective binding reduces the ability of BoNT/A to bind to said SV2, either by preventing BoNT/A binding to a BoNT/A receptor, or by preventing said SV2 association with a FGFR3. Other aspect of the present invention provide methods of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2 complex, a FGFR3 and a SV2 complex, or a FGFR2, a FGFR3 and a SV2 complex, wherein said selective binding reduces the ability of BoNT/A to bind to said FGFR2, either by preventing BoNT/A binding to a BoNT/A receptor, or by preventing said SV2 association with a FGFR2.

[021] Other aspect of the present invention provide methods of screening for a molecule able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication by contacting said sample with a composition comprising a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2 complex, a FGFR3 and a SV2 complex, or a FGFR2, a FGFR3 and a SV2 complex, and detecting whether said molecule selectively binds said FGFR2, said FGFR3, said SV2, said FGFR2/SV2 complex, said FGFR3/SV2 complex, or said FGFR2/FGFR3/SV2 complex, wherein selective binding of said molecule to said FGFR2, said FGFR3, said SV2, said FGFR2/SV2 complex, said FGFR3/SV2 complex, or said FGFR2/FGFR3/SV2 complex indicates that said molecule is able to compete with BoNT/A for selective

binding to cells susceptible to BoNT/A intoxication, and wherein if said molecule is BoNT/A, said method does not comprise an LD₅₀ assay.

[022] Other aspect of the present invention provide methods of marketing a neurotoxin capable of selectively binding to the same FGFR2, FGFR3, SV2, or any combination thereof, as BoNT/A comprising obtaining marketing approval from a governmental or regional regulatory authority for a therapeutic neurotoxin, wherein said neurotoxin is assayed for selective binding to a cell comprising contacting said neurotoxin with a composition comprising a FGFR2, a FGFR3, a SV2, or any combination thereof, and detecting whether said neurotoxin selectively binds said FGFR2, said FGFR3, said SV2, or any combination thereof, wherein selective binding of said neurotoxin to said FGFR2, said FGFR3, said SV2, or any combination thereof, indicates that said neurotoxin is able to selective binding to cells susceptible to BoNT/A intoxication and wherein if said molecule is BoNT/A, said method does not comprise an LD₅₀ assay; packaging said neurotoxin for sale in a manner consistent with the requirements of said regulatory authority, and selling said neurotoxin.

[023] Other aspect of the present invention provide methods of marketing a neurotoxin capable of selectively binding to the same FGFR2, FGFR3, SV2, or any combination thereof, as BoNT/A comprising obtaining marketing approval from a governmental or regional regulatory authority for a therapeutic neurotoxin, wherein said neurotoxin is assayed for selective binding to a cell comprising contacting said neurotoxin to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity; packaging said neurotoxin for sale in a manner consistent with the requirements of said regulatory authority, and selling said neurotoxin.

[024] BoNTs are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulphide loop by bacterial or tissue proteases. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus; 2) a translocation domain (H_N) contained within the amino-terminal half of the heavy chain (HC) that facilitates release of the toxin from intracellular vesicles into the cytoplasm of the target cell; and 3) a binding domain (H_C) found within the carboxyl-terminal half of the HC that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell.

[025] The binding, translocation and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby BoNTs enter a neuron and inhibit neurotransmitter release is similar, regardless of type. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising four steps: 1) receptor binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (see FIG. 1). The process is initiated when the H_C domain of a BoNT binds to BoNT-specific receptor complex located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each BoNT/A receptor complex. Once bound, the BoNT/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements of the toxin: 1) increased hydrophobicity of the translocation domain which facilitates the formation of a pore in the intracellular vesicle, and 2) di-chain formation of the toxin which enhances enzymatic activity of the light chain endopeptidase. Once detached from the heavy chain, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it specifically targets one of three known core components of the neurotransmitter release apparatus. Three of these core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble N-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. The selective proteolysis of synaptic SNAREs accounts for the total block of neurotransmitter release caused by clostridial toxins *in vivo*. The SNARE protein targets of clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, e.g., Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release*, 82(5) *Biochimie*. 427-446 (2000); Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility*, 27(11) *Trends Biochem. Sci.* 552-558. (2002); M. Zouhair Atassi, *Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins*, (Dirk W. Dressler & Joseph J. Jankovic eds., 2003); Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons*, 11(9) *Trends Microbiol.* 431-437, (2003).

[026] The three-dimensional crystal structures of BoNT/A indicate that the three functional domains of the toxin are structurally distinct, see e.g., Humeau et al., *supra*, (2000), Turton et al., *supra*, (2002); and Lalli et al., *supra*, (2003). The HEXXH consensus motif of the light chain forms the tetrahedral zinc binding pocket of the catalytic site located in a deep cleft on the protein surface that is accessible by a channel. This conserved zinc binding motif binds at least one zinc atom necessary for its catalytic function. The structure of the H_N and H_C domains consists primarily of β -sheet topologies that are linked by a single α -helix. The H_N domain comprises a β -barrel, jelly-roll fold that resembles the carbohydrate binding moiety found in lectins suggesting that this domain may recognize oligosaccharide-containing molecules and play a role in the intracellular sorting. In addition to its overall structural similarity with

lectins, the H_N domain also contains two distinct structural features suggesting functions. First, the H_N domain contains a pair of long amphipathic helices that resemble the coiled-coil motif found in some viral proteins. In viruses, these helices assist in fusing the viral membrane to the cellular membrane of the host, suggesting that the coiled-coil region may assist in inserting the H_N domain into the membrane of an intracellular vesicle. Second, a long loop called the 'translocation belt,' wraps around a large negatively charged cleft of the light chain that blocks access of the zinc atom to the catalytic-binding pocket of active site. The H_C domain contains a ganglioside-binding site and a five residue ganglioside-binding motif. These regions adopt a modified β -trefoil fold structure which forms four distinct carbohydrate binding regions believed to mediate the binding to specific carbohydrate containing acceptor molecules on the cell surface. Consistent with this function, the H_C domain exhibits the highest sequence divergence between clostridial toxins which may account for the distinct binding properties and sorting schemes of TeNT and BoNTs. The H_C domain tilts away from the H_N domain exposing the surface loops and making them accessible for binding. No contact seems to occur between the light chain and the H_C domain. The N-terminus of the H_C region presents a jelly-roll architecture related to that of the S-lectins, a carbohydrate-binding family of proteins. By contrast, the C-terminus of H_C is in a pseudo threefold trefoil conformation that presents structural similarity to the sequentially unrelated interleukins-1 α and 1 β , Kunitz-type trypsin inhibitors, as well as fibroblast growth factors (FGF). These proteins, mostly β -proteins, are involved in protein-protein interactions.

[027] Cell surface gangliosides appear to be part of the receptor system for BoNT/A and seem to participate in binding of the toxin to its BoNT/A receptor. Although toxin binding is not strictly dependent on the presence of gangliosides, the presence of specific gangliosides appears to be required for high affinity binding. In particular, BoNTs have been observed to interact *in vitro* and *in vivo* with polysialogangliosides, especially those of the G1b series (GD1a, GD1b, GD3, GQ1b, or GT1b), see, e.g., Jane L. Halpern & Elaine A. Neale, *Neurospecific Binding, Internalization, and Retrograde Axonal Transport*, 195 Curr. Top. Microbiol. Immunol. 221-241 (1995). Preincubation of the toxin with these gangliosides protects the neuromuscular junction (NMJ) of mice from BoNT toxicity. High-affinity, trypsin-sensitive, BoNT-binding sites were found in isolated synaptosomes, see, e.g., R. S. Williams et al, *Radioiodination of Botulinum Neurotoxin Type A with Retention of Biological Activity and its Binding to Brain Synaptosomes*, 131(2) Eur. J. Biochem. 1437-1445 (1983). Since lectins with high affinity for sialic acid antagonize the binding of BoNTs, their protein receptors may be glycoproteins. Receptors for BoNTs would direct them to acidic vesicles allowing the translocation of the LC into the cytosol of the neuron. The amino acid sequence at the C-terminus of H_C is poorly conserved among different clostridial neurotoxins, and competition experiments have shown that different BoNT serotypes bind to different protein receptors on the surface of neuronal cells. This analysis is therefore consistent with the hypothesis that BoNTs neurotoxins bind to receptor systems comprising at least two components; a protein component and a carbohydrate component.

[028] Based on these findings, and as the present disclosure provides herein, the Applicants have discovered that cells expressing a FGFR2 or a FGFR3, or both a FGFR2 and a FGFR3, can bind

BoNT/A. Internalization of the toxin can be followed when these cell lines are exposed to the toxin. Moreover, BoNT/A internalization is inhibited in a dose-dependent manner when FGF, such as, e.g., FGF1, FGF2, FGF4, FGF8 and FGF9, is added at increasing concentrations. Cells tested by the Applicants that did not display FGFR3 were unable to internalize the toxin, although when subjected to electroporation in the presence of BoNT/A, the intracellular cleavage of SNAP-25 could be detected, indicating that the endopeptidase activity of the toxin remained intact, and that the cells remained susceptible to the endopeptidase. In addition, the Applicants have found that pre-treatment with the polysialoganglioside GT1b increases BoNT/A cellular uptake.

[029] Fibroblast growth factors (FGF) participate in many developmental, differentiation and growth and repair processes of cells through complex combinatorial signaling pathways. Presently, at least 23 ligands (FGF1-23) are known to signal through a family of five transmembrane tyrosine kinase FGF receptors (FGFR1-5). The amino acid sequence identity is highly conserved between FGFR family members and each share a characteristic structural organization. The extracellular portion of FGFRs comprise an amino-terminal hydrophobic signal peptide, three Ig-like domains (IgI, IgII and IgIII) and an acid box domain of approximately eight acidic residues, followed by a single hydrophobic transmembrane domain, which in turn is followed by an intracellular tyrosine kinase domain (see FIG. 2). Affinity of FGFRs for their ligands is highly diverse with different affinities for each family member of growth factors, see, e.g., C. J. Powers et al., *Fibroblast Growth Factors, Their Receptors and Signaling*, 7(3) *Endocr. Relat. Cancer*. 165-197 (2000). Table 1 lists some of the known FGF-FGFR signaling relationships of various FGFs and their FGFRs.

TABLE 1. FGFR Variants								
Variant	FGFR1		FGFR2		FGFR3		FGFR4	FGFR5
	IIIb	IIIc	IIIb	IIIc	IIIb	IIIc		
Ligands	FGF-1 FGF-2 FGF-3 FGF-8 FGF-10	FGF-1 FGF-2 FGF-4 FGF-5 FGF-6 FGF-8 FGF-17	FGF-1 FGF-3 FGF-7 FGF-10	FGF-1 FGF-2 FGF-4 FGF-5 FGF-6 FGF-8 FGF-9 FGF-17	FGF-1 FGF-9	FGF-1 FGF-2 FGF-4 FGF-8 FGF-9	FGF-1 FGF-2 FGF-4 FGF-6 FGF-8 FGF-9	FGF-1 FGF-2
Tissues	Brain, bone, kidney, skin, lung, heart, muscle, neuron	Brain, kidney, skin, lung, liver, glial cells	Brain, CNS, kidney, skin, lung, testis	Lung, liver, kidney	Brain, skin, lung, testis			
FGFR variants, associated ligands, and tissue distribution, see, e.g., . Powers et al, <i>supra</i> , (2000); and Reuss & von Bohlen und Halbach, <i>supra</i> , (2003).								

[030] Diversity in FGF signaling beyond the five receptors is achieved in part by the generation of alternatively spliced variants encoding distinct receptor isoforms, see, e.g., Bernhard Reuss & Oliver von Bohlen und Halbach, *Fibroblast Growth Factors and Their Receptors in the Central Nervous System*,

313(2) Cell Tissue Res. 139-157 (2003). The protein region that appears to have the highest influence on ligand binding specificity is a portion of the IgIII domain, for which isoforms encoded by three different splice variants have been identified. These three isoforms, designated IgIIIa, IgIIIb and IgIIIc, have different binding affinities for different FGFR family members. Alternative splicing in the FGFR ligand binding domain, designated a and b, generates additional receptor isoforms with novel ligand affinities. Isoforms for IgIIIa, IgIIIb and IgIIIc have been identified for both FGFR1 and FGFR2. Thus far, the IgIIIa isoform of FGFR3 and the IgIIIa and IgIIIb isoforms of FGFR4 and FGFR5 have not been reported.

[031] As mentioned above, FGFR3 commonly exists in two isoforms, FGFR3IIIc and FGFR3IIIb, which arise following alternative splicing of the primary transcript in which either exon 8 or 9 respectively is skipped (see FIG. 2). However, additional isoforms exist. For example, an FGFR3 isoform has been described which lacks the acid box, see, e.g., Akio Shimizu et al, *A Novel Alternatively Spliced Fibroblast Growth Factor Receptor 3 Isoform Lacking the Acid Box Domain is Expressed During Chondrogenic Differentiation of ATDC5 Cells*, 276(14) J. Biol. Chem. 11031-11040 (2001). In another example, a novel, secreted isoform was recently identified, called FGFR3S, in which exons 8, 9 and 10 are spliced out creating a FGFR3 that lacks the second half of IgIIIc and the transmembrane domain, see, e.g., L-M. Sturla et al., *FGFR3IIS: A Novel Soluble FGFR3 Spliced Variant That Modulates Growth is Frequently Expressed in Tumor Cells*, 89(7) Br. J. Cancer 1276-1284 (2003).

[032] Aspects of the present invention provide, in part, a method of detecting BoNT/A activity by contacting a sample to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another embodiment a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[033] As used herein, the term "exogenous FGFR2" refers to a FGFR2 expressed in a cell through the introduction of an external source of FGFR2 or an external source of genetic material encoding a FGFR2 by human manipulation. As used herein, the term "endogenous FGFR2" refers to a FGFR2 naturally present in the cell because it is naturally encoded within the cell's genome, such that the cell inherently expresses the FGFR2 without the need an external source of FGFR2 or an external source of genetic

material encoding a FGFR2. As used herein, the term “exogenous FGFR3” refers to a FGFR3 expressed in a cell through the introduction of an external source of FGFR3 or an external source of genetic material encoding a FGFR3 by human manipulation. As used herein, the term “endogenous FGFR3” refers to a FGFR3 naturally present in the cell because it is naturally encoded within the cell's genome, such that the cell inherently expresses the FGFR3 without the need an external source of FGFR3 or an external source of genetic material encoding a FGFR3. As used herein, the term “exogenous SV2” refers to a SV2 expressed in a cell through the introduction of an external source of FGFR3 or an external source of genetic material encoding a SV2 by human manipulation. As used herein, the term “endogenous SV2” refers to a SV2 naturally present in the cell because it is naturally encoded within the cell's genome, such that the cell inherently expresses the SV2 without the need an external source of SV2 or an external source of genetic material encoding a SV2.

[034] As used herein “botulinum toxin serotype A” is synonymous with “BoNT/A,” “type A,” or similar terminology referring unambiguously to *Clostridium botulinum* neurotoxin type A, means any of a number of polypeptide neurotoxins, and derivatives thereof, which can be purified from *Clostridium botulinum* serotype A strains and which share FGFR2, FGFR3, and/or SV2 as cell surface receptors. As a non-limiting example, there are presently five BoNT/A subtypes, BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific subtypes showing approximately 89% amino acid identity when compared to another BoNT/A subtype. Such neurotoxins include, without limitation, those found in or corresponding to the following strains and accession numbers listed in Table 2.

TABLE 2	
Strain	Accession No.
CL138	AAQ16535
137	AAQ16534
129	AAQ16533
13	AAQ16532
42N	AAQ16531
Hall A-hyper	AAM75961
667Ab	CAA61124
NCTC 2916	CAA36289
Allergan-Hall A	AAQ06331
62A	AAA23262
Kyoto-F	CAA51824
Type A NIH NCTC 7272 7103-H	BAA11051
Kumgo	AAO21363

[035] As used herein, the term “Fibroblast Growth Factor 3 Receptor” is synonymous with “FGFR3” and means a FGFR3 peptide or peptidomimetic which binds BoNT/A in a manner that elicits a BoNT/A

intoxication response. FGFR3s useful in the invention encompass, without limitation, wild type FGFR3s, naturally occurring FGFR3 variants, non-naturally FGFR3 variants, such as, e.g., genetically engineered variants produced by random mutagenesis or rationally designed, and active fragments derived from a FGFR3s. As a non-limiting example, a human FGFR3, naturally occurring human FGFR3 variants, non-naturally human FGFR3 variants, and human FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a bovine FGFR3, naturally occurring bovine FGFR3 variants, non-naturally bovine FGFR3 variants, and bovine FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a rat FGFR3, naturally occurring rat FGFR3 variants, non-naturally rat FGFR3 variants, and rat FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In still another non-limiting example, a mouse FGFR3, naturally occurring mouse FGFR3 variants, non-naturally mouse FGFR3 variants, and mouse FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a chicken FGFR3, naturally occurring chicken FGFR3 variants, non-naturally chicken FGFR3 variants, and chicken FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a frog FGFR3, naturally occurring frog FGFR3 variants, non-naturally frog FGFR3 variants, and frog FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a newt FGFR3, naturally occurring newt FGFR3 variants, non-naturally newt FGFR3 variants, and newt FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a zebrafish FGFR3, naturally occurring zebrafish FGFR3 variants, non-naturally zebrafish FGFR3 variants, and zebrafish FGFR3 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. It is also understood that both nucleic acid molecules, such as, e.g., DNA and RNA, that encode a FGFR3 disclosed in the present specification and peptide molecules or peptidomimetics comprising a FGFR3 disclosed in the present specification are useful in aspects of the present invention. SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27 disclose nucleic acid molecules encoding representative of FGFR3s useful in aspects on the present invention, while SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 disclose peptide molecules representative of FGFR3s useful in aspects on the present invention.

[036] As used herein, the term “peptidomimetic” is used broadly to mean a peptide-like molecule that selectively binds BoNT/A as the peptide BoNT/A receptor upon which it is structurally based. Such peptidomimetics include chemically modified peptides, peptide-like molecules containing non-naturally occurring amino acids, and peptoids, which are peptide-like molecules resulting from oligomeric

assembly of N-substituted glycines, and selectively bind BoNT/A as the peptide substrate upon which the peptidomimetic is derived, see, e.g., Goodman and Ro, *Peptidomimetics for Drug Design*, in "Burger's Medicinal Chemistry and Drug Discovery" Vol. 1 (ed. M.E. Wolff; John Wiley & Sons 1995), pages 803-861).

[037] A variety of peptidomimetics are known in the art including, for example, peptide-like molecules which contain a constrained amino acid, a non-peptide component that mimics peptide secondary structure, or an amide bond isostere. A peptidomimetic that contains a constrained, non-naturally occurring amino acid can include, e.g., an α -methylated amino acid; an α,α -dialkyl-glycine or α -aminocycloalkane carboxylic acid; an N^α -C $^\alpha$ cyclized amino acid; an N^α -methylated amino acid; a β - or γ -amino cycloalkane carboxylic acid; an α,β -unsaturated amino acid; a β , β -dimethyl or β -methyl amino acid; a β -substituted-2,3-methano amino acid; an NC $^\delta$ or C $^\alpha$ -C $^\delta$ cyclized amino acid; or a substituted proline or another amino acid mimetic. In addition, a peptidomimetic which mimics peptide secondary structure can contain, e.g., a nonpeptidic β -turn mimic; γ -turn mimic; mimic of β -sheet structure; or mimic of helical structure, each of which is well known in the art. A peptidomimetic also can be a peptide-like molecule which contains, e.g., an amide bond isostere such as a retro-inverso modification; reduced amide bond; methylenethioether or methylenesulfoxide bond; methylene ether bond; ethylene bond; thioamide bond; trans-olefin or fluoroolefin bond; 1,5-disubstituted tetrazole ring; ketomethylene or fluoroketomethylene bond or another amide isostere. One skilled in the art understands that these and other peptidomimetics are encompassed within the meaning of the term "peptidomimetic" as used herein.

[038] Thus, in aspects of this embodiment, the FGFR3 can be a FGFR3IIIb that selectively binds BoNT/A having amino acid identity that is, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% the amino acid identity of SEQ ID NO: 2, SEQ ID NO: 10, or SEQ ID NO: 16. In other aspects of this embodiment, a FGFR3IIIb that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 2, SEQ ID NO: 10, or SEQ ID NO: 16. In other aspects of this embodiment, a FGFR3IIIb that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 2, SEQ ID NO: 10, or SEQ ID NO: 16. In other aspects of this embodiment, a FGFR3IIIb that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 2, SEQ ID NO: 10, or SEQ ID NO: 16. In other aspects of this embodiment, a FGFR3IIIb that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 2, SEQ ID NO: 10, or SEQ ID NO: 16.

[039] In other aspects of this embodiment, the FGFR3 can be a FGFR3IIIc that selectively binds BoNT/A having amino acid identity that is, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% the amino acid identity of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 18. In other aspects of this embodiment, a FGFR3IIIc that selectively binds a BoNT/A has,

e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 18. In other aspects of this embodiment, a FGFR3IIIc that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 18. In other aspects of this embodiment, a FGFR3IIIc that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 18. In other aspects of this embodiment, a FGFR3IIIc that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, or SEQ ID NO: 18.

[040] In other aspects of this embodiment, the FGFR3 can be a FGFR3IIIS that selectively binds BoNT/A having amino acid identity that is, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% the amino acid identity of SEQ ID NO: 6. In other aspects of this embodiment, a FGFR3IIIS that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a FGFR3IIIS that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a FGFR3IIIS that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 6. In other aspects of this embodiment, a FGFR3IIIS that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 6.

[041] In other aspects of this embodiment, the FGFR3 can be a FGFR3-delAcid that selectively binds BoNT/A having amino acid identity that is, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% the amino acid identity of SEQ ID NO: 14. In other aspects of this embodiment, a FGFR3-delAcid that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 14. In other aspects of this embodiment, a FGFR3-delAcid that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 14. In other aspects of this embodiment, a FGFR3-delAcid that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 14. In other aspects of this embodiment, a FGFR3-delAcid that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 14.

[042] In other aspects of this embodiment, the FGFR3 can be a FGFR3 that selectively binds BoNT/A having amino acid identity that is, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% the amino acid identity of SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID

NO: 26, or SEQ ID NO: 28. In other aspects of this embodiment, a FGFR3 that selectively binds a BoNT/A has, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28. In other aspects of this embodiment, a FGFR3 that selectively binds a BoNT/A has, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28. In other aspects of this embodiment, a FGFR3 that selectively binds a BoNT/A has, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28. In other aspects of this embodiment, a FGFR3 that selectively binds a BoNT/A has, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, or SEQ ID NO: 28.

[043] As used herein, the term “Fibroblast Growth Factor 2 Receptor” is synonymous with “FGFR2” and means a FGFR2 peptide or peptidomimetic which binds BoNT/A in a manner that elicits a BoNT/A intoxication response. FGFR2s useful in the invention encompass, without limitation, wild type FGFR2s, naturally occurring FGFR2 variants, non-naturally FGFR2 variants, such as, *e.g.*, genetically engineered variants produced by random mutagenesis or rational designed, and active fragments derived from a FGFR2. As a non-limiting example, a human FGFR2, naturally occurring human FGFR2 variants, non-naturally human FGFR2 variants, and human FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a bovine FGFR2, naturally occurring bovine FGFR2 variants, non-naturally bovine FGFR2 variants, and bovine FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a rat FGFR2, naturally occurring rat FGFR2 variants, non-naturally rat FGFR2 variants, and rat FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In still another non-limiting example, a mouse FGFR2, naturally occurring mouse FGFR2 variants, non-naturally mouse FGFR2 variants, and mouse FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a chicken FGFR2, naturally occurring chicken FGFR2 variants, non-naturally chicken FGFR2 variants, and chicken FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a frog FGFR2, naturally occurring frog FGFR2 variants, non-naturally frog FGFR2 variants, and frog FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a newt FGFR2, naturally occurring newt FGFR2 variants, non-naturally newt FGFR2 variants, and newt FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a

zebrafish FGFR2, naturally occurring zebrafish FGFR2 variants, non-naturally zebrafish FGFR2 variants, and zebrafish FGFR2 fragments that retain the ability to selectively bind BoNT/A and mediate the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. It is also understood that both nucleic acid molecules, such as, e.g., DNA and RNA, that encode a FGFR2 disclosed in the present specification and peptide molecules or peptidomimetics comprising a FGFR2 disclosed in the present specification are useful in aspects of the present invention. SEQ ID NO: 37, 39, 41, 43, 45, 47, 49, 51, and 53 disclose nucleic acid molecules encoding representative of FGFR2s useful in aspects on the present invention, while SEQ ID NO: 38, 40, 42, 44, 46, 48, 50, 52, 54, 55, 56, and 57 disclose peptide molecules representative of FGFR2s useful in aspects on the present invention.

[044] In aspects of this embodiment, a FGFR2 that selectively binds a BoNT/A has amino acid identity that is, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% the amino acid identity of SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, or SEQ ID NO: 57.

[045] In other aspects of this embodiment, a FGFR2 that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, or SEQ ID NO: 57. In other aspects of this embodiment, a FGFR2 that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, or SEQ ID NO: 57.

[046] In other aspects of this embodiment, a FGFR2 that selectively binds a BoNT/A has, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, or SEQ ID NO: 57. In other aspects of this embodiment, a FGFR2 that selectively binds a BoNT/A has, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, or SEQ ID NO: 57.

[047] Aspects of the present invention provide, in part, a method of detecting BoNT/A activity by contacting a sample to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to

said control cell is indicative of BoNT/A activity. In an embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another embodiment a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[048] As used herein, the term “synaptic vesicle glycoprotein 2” is synonymous with “SV2” and means a SV2 peptide or peptidomimetic which associates with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby eliciting a BoNT/A intoxication response. SV2s useful in the invention encompass, without limitation, wild type SV2s, naturally occurring SV2s variants, non-naturally SV2s variants, such as, *e.g.*, genetically engineered variants produced by random mutagenesis or rational designed, and active fragments derived from a SV2s. In addition, any SV2 which associates with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby eliciting a BoNT/A intoxication response can be useful in practicing aspect of this invention, including, without limitation, vertebrate SV2s, such as, *e.g.*, mammalian SV2s, bird SV2s, amphibian SV2s, and fish SV2s, and invertebrate SV2s, such as, *e.g.*, insect SV2s and worm SV2s. As a non-limiting example, a human SV2, naturally occurring human SV2 variants, non-naturally human SV2 variants, and human SV2 fragments that retain the ability to selectively associate with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby mediating the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a primate SV2, naturally occurring primate SV2 variants, non-naturally primate SV2 variants, and primate SV2 fragments that retain the ability to selectively associate with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby mediating the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a bovine SV2, naturally occurring bovine SV2 variants, non-naturally bovine SV2 variants, and bovine SV2 fragments that retain the ability to selectively associate with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby mediating the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a rat SV2, naturally occurring rat SV2 variants, non-naturally rat SV2 variants, and rat SV2 fragments that retain the ability to selectively associate with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby mediating the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention. In another non-limiting example, a mouse SV2, naturally

occurring mouse SV2 variants, non-naturally mouse SV2 variants, and mouse SV2 fragments that retain the ability to selectively associate with FGFR2 and/or FGFR3 in a manner that enhances BoNT/A binding, thereby mediating the intoxication process, can be useful as a BoNT/A receptor in aspects of the present invention.

[049] Cell compositions comprising a FGFR2 and a SV2, a FGFR3 and a SV2, or a FGFR2, a FGFR3 and a SV2 can enhance the selective binding of BoNT/A relative to a composition not containing a SV2. Non-limiting examples of a SV2 include a SV2A, a SV2B, and a SV2C. Other non-limiting examples of a SV2 include SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36. Thus, in an embodiment, a composition comprises a FGFR2 and a SV2. In aspects of this embodiment, a composition comprises a FGFR2 and a SV2, such as, *e.g.*, a SV2A, a SV2B, a SV2C, or any combination thereof. In aspects of this embodiment, a composition comprises a FGFR2 and a SV2, such as, *e.g.*, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, or any combination thereof. In another embodiment, a composition comprises a FGFR3 and a SV2. In aspects of this embodiment, a composition comprises a FGFR3 and a SV2, such as, *e.g.*, a SV2A, a SV2B, a SV2C, or any combination thereof. In aspects of this embodiment, a composition comprises a FGFR3 and a SV2, such as, *e.g.*, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, or any combination thereof. In yet another embodiment, a composition comprises a FGFR2, a FGFR3 and a SV2. In aspects of this embodiment, a composition comprises a FGFR2, a FGFR3 and a SV2, such as, *e.g.*, a SV2A, a SV2B, a SV2C, or any combination thereof. In aspects of this embodiment, a composition comprises a FGFR2, a FGFR3 and a SV2, such as, *e.g.*, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, or any combination thereof.

[050] Thus, in an embodiment, the methods disclosed in the present invention comprise a SV2 that enhances the selective binding of BoNT/A. In aspects of this embodiment, a SV2 that enhances the selective binding of BoNT/A has an amino acid identity that is, *e.g.*, at least 70%, at least 75%, at least 80%, at least 90%, or at least 95% of SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36.

[051] In other aspects of this embodiment, a SV2 that enhances the selective binding of BoNT/A has, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36. In other aspects of this embodiment, a SV2 that enhances the selective binding of BoNT/A has, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36.

[052] In other aspects of this embodiment, a SV2 that enhances the selective binding of BoNT/A has, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 contiguous amino acid substitutions, deletions and/or additions relative to SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36. In other aspects of this embodiment, a SV2 that enhances the selective binding of BoNT/A has, *e.g.*, at most 1, 2, 3, 4, 5, 6, 7, 8,

9, or 10 contiguous amino acid substitutions, deletions, and/or additions relative to SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36.

[053] Other aspects of the present invention provide, in part, the optional use of a polysialogangliosides, especially those of the G1b series, such as, *e.g.*, GD1a, GD1b, GD3, GQ1b, or GT1b. Cell compositions comprising a FGFR3 and a polysialoganglioside can increase the selective binding of BoNT/A relative to a composition not containing a polysialoganglioside. Thus, in an embodiment, a composition comprises a FGFR3 and optionally a polysialoganglioside. In aspects of this embodiment, a composition comprises a FGFR3 and optionally a G1b polysialoganglioside, such as, *e.g.*, GD1a, GD1b, GD3, GQ1b, or GT1b.

[054] Thus, in an embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, and optionally a G1b polysialoganglioside wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, and a G1b polysialoganglioside wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another embodiment a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, and a G1b polysialoganglioside wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[055] Other aspects of the present invention provide, in part, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. Other aspects of the present invention provide, in part, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. As used herein, the term “transiently containing” means a FGFR2, a FGFR3, a SV2, or any combination thereof, that is temporarily introduced into a cell in order

to perform the assays disclosed in the present specification. Thus, aspects of a cell transiently containing a FGFR2, a FGFR3, a SV2, or any combination thereof disclosed in the specification can include a cell that contains a FGFR2, a FGFR3, a SV2, or any combination thereof, for, e.g., at most about one day, at most about two days, at most about three days, at most about four days, at most about five days, and at most about six days, at most about seven days, at most about eight days, at most about nine days and at most about ten days.

[056] In an aspect of this embodiment, the FGFR3 can be encoded by the nucleic acid molecule from a mammalian FGFR3, such as, e.g., a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3; a bird FGFR3, such as, e.g., chicken FGFR3; an amphibian FGFR3, such as, e.g., a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, e.g., a zebrafish FGFR3. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous mammalian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous bird FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous amphibian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous fish FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[057] In another aspect of this embodiment, the FGFR3 can be a mammalian FGFR3, such as, e.g., a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3; a bird FGFR3, such as, e.g., chicken FGFR3; an amphibian FGFR3, such as, e.g., a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, e.g., a zebrafish FGFR3. Thus, in an embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In aspect of this embodiment, the FGFR3 can be a mammalian FGFR3, such as, e.g., a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3;

a bird FGFR3, such as, e.g., chicken FGFR3; an amphibian FGFR3, such as, e.g., a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, e.g., a zebrafish FGFR3. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous mammalian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous bird FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous amphibian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous fish FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[058] In an aspect of this embodiment, the FGFR2 can be encoded by the nucleic acid molecule from a mammalian FGFR2, such as, e.g., a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, e.g., chicken FGFR2; an amphibian FGFR2, such as, e.g., a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, e.g., a zebrafish FGFR2. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous mammalian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous bird FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding an exogenous amphibian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that

transiently contains a nucleic acid molecule encoding an exogenous fish FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[059] In another aspect of this embodiment, the FGFR2 can be a mammalian FGFR2, such as, *e.g.*, a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, *e.g.*, chicken FGFR2; an amphibian FGFR2, such as, *e.g.*, a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, *e.g.*, a zebrafish FGFR2. Thus, in an embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In aspect of this embodiment, the FGFR2 can be a mammalian FGFR2, such as, *e.g.*, a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, *e.g.*, chicken FGFR2; an amphibian FGFR2, such as, *e.g.*, a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, *e.g.*, a zebrafish FGFR2. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous mammalian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous bird FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous amphibian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous fish FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[060] In an aspect of this embodiment, a SV2 can be encoded by the nucleic acid molecule, such as, *e.g.*, those encoding for a SV2A, a SV2B, or a SV2C. In other aspects of this embodiment, a SV2 can be encoded by the nucleic acid molecule, such as, *e.g.*, those encoding for SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains a nucleic acid molecule encoding

an exogenous SV2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[061] In another aspect of this embodiment, a SV2 can be, such as, *e.g.*, a SV2A, a SV2B or a SV2C. In other aspects of this embodiment, a SV2 can be, such as, *e.g.*, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that transiently contains an exogenous SV2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[062] Other aspects of the present invention provide, in part, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. Other aspect of the present invention provide, in part, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. As used herein, the term “stably containing” means a FGFR2, a FGFR3, a SV2, or any combination thereof, that is introduced into a cell and maintained for long periods of time in order to perform the assays of the present specification. Stably-maintained nucleic acid molecules encompass stably-maintained nucleic acid molecules that are extra-chromosomal and replicate autonomously and stably-maintained nucleic acid molecules that are integrated into the chromosomal material of the cell and replicate non-autonomously. Thus aspects of a cell stably containing a FGFR2, a FGFR3, a SV2, or any combination thereof, disclosed in the specification may include a cell that contains a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2, a FGFR3 and a SV2, or a FGFR2, a FGFR3 and a SV2 for, *e.g.*, at least ten days, at least 20 two days, at least 30 days, at least forty days, at least 50 days, and at least 60 days, at least 70 days, at least 80 days, at least 90 days and at least 100 days. Other aspects of a cell stably containing a FGFR2, a FGFR3, a SV2, or any combination thereof, disclosed in the specification may include a cell that contains a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2, a FGFR3 and a SV2, or a FGFR2, a FGFR3 and a SV2 for, *e.g.*, at least 100 days, at least 200 days, at least 300 days, at least 400 days, and at least 500 days. Still other aspects of a cell stably containing a FGFR3, a SV2, a FGFR2 or both a FGFR3 and a SV2, both a FGFR2 and a SV2, both a FGFR3 and a FGFR2, or a FGFR2 and a FGFR3 and a SV2 disclosed in the specification may include a cell that permanently contains a FGFR3, a SV2, a FGFR2, or both a FGFR3 and a SV2, both a FGFR2 and a SV2, both a FGFR3 and a FGFR2, or a FGFR2 and a FGFR3 and a SV2

[063] In an aspect of this embodiment, the FGFR2 can be encoded by the nucleic acid molecule from a mammalian FGFR2, such as, e.g., a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, e.g., chicken FGFR2; an amphibian FGFR2, such as, e.g., a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, e.g., a zebrafish FGFR2. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous mammalian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous bird FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous amphibian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous fish FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[064] In another aspect of this embodiment, the FGFR2 can be a mammalian FGFR2, such as, e.g., a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, e.g., chicken FGFR2; an amphibian FGFR2, such as, e.g., a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, e.g., a zebrafish FGFR2. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous mammalian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous bird FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous amphibian FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said

contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous fish FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[065] In an aspect of this embodiment, the FGFR3 can be encoded by the nucleic acid molecule from a mammalian FGFR3, such as, *e.g.*, a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3; a bird FGFR3, such as, *e.g.*, chicken FGFR3; an amphibian FGFR3, such as, *e.g.*, a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, *e.g.*, a zebrafish FGFR3. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous mammalian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous bird FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous amphibian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous fish FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[066] In another aspect of this embodiment, the FGFR3 can be a mammalian FGFR3, such as, *e.g.*, a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3; a bird FGFR3, such as, *e.g.*, chicken FGFR3; an amphibian FGFR3, such as, *e.g.*, a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, *e.g.*, a zebrafish FGFR3. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous mammalian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably

contains an exogenous bird FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous amphibian FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous fish FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[067] In an aspect of this embodiment, a SV2 can be encoded by the nucleic acid molecule, such as, e.g., those encoding for a SV2A, a SV2B, or a SV2C. In other aspects of this embodiment, a SV2 can be encoded by the nucleic acid molecule, such as, e.g., those encoding for SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36. In an aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains a nucleic acid molecule encoding an exogenous SV2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[068] In another aspect of this embodiment, a SV2 can be, such as, e.g., a SV2A, a SV2B, or a SV2C. In another aspect of this embodiment, a SV2 can be, such as, e.g., SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36. In another aspect of this embodiment, a method of detecting BoNT/A activity comprises contacting a sample to a cell that stably contains an exogenous SV2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

[069] As mentioned above, a nucleic acid molecule can be used to express a FGFR2, a FGFR3 and/or a SV2 disclosed in the present specification. It is envisioned that any and all methods for introducing a nucleic acid molecule into a cell can be used. Methods useful for introducing a nucleic acid molecule into a cell including, without limitation, calcium phosphate-mediated, DEAE dextran-mediated, lipid-mediated, polybrene-mediated, polylysine-mediated, viral-mediated, microinjection, protoplast fusion, biolistic, electroporation and conjugation to an antibody, gramicidin S, artificial viral envelope or other intracellular carrier such as TAT., see, e.g., *Introducing Cloned Genes into Cultured Mammalian Cells*, pp. 16.1-16.62 (Sambrook & Russell, eds., *Molecular Cloning A Laboratory Manual*, Vol. 3, 3rd ed. 2001); Alessia Colosimo et al., *Transfer and Expression of Foreign Genes in Mammalian Cells*, 29(2) *Biotechniques* 314-318, 320-322, 324 (2000); Philip Washbourne & A. Kimberley McAllister, *Techniques for Gene*

Transfer into Neurons, 12(5) Curr. Opin. Neurobiol. 566-573 (2002); and Current Protocols in Molecular Biology, John Wiley and Sons, pp 9.16.4-9.16.11 (2000). One skilled in the art understands that selection of a specific method to introduce a nucleic acid molecule into a cell will depend, in part, on whether the cell will transiently contain a BoNT/A receptor or whether the cell will stably contain a BoNT/A receptor.

[070] As mentioned above, a FGFR2, a FGFR3 and/or SV2 disclosed in the present specification can be introduced into a cell. It is envisioned that any and all methods using a delivery agent to introduce a FGFR3 and/or a SV2 into a cell can be used. As used herein, the term "delivery agent" means any molecule that enables or enhances internalization of a covalently-linked, non-covalently-linked or in any other manner associated with a FGFR3 and/or a SV2 into a cell. Thus, the term "delivery agent" encompasses, without limitation, proteins, peptides, peptidomimetics, small molecules, nucleic acid molecules, liposomes, lipids, viruses, retroviruses and cells that, without limitation, transport a covalently or non-covalently linked substrate to the cell membrane, cell cytoplasm or nucleus. It further is understood that the term "delivery agent" encompasses molecules that are internalized by any mechanism, including delivery agents which function via receptor mediated endocytosis and those which are independent of receptor mediated endocytosis.

[071] A delivery agent useful in the invention also can be an agent that enables or enhances cellular uptake of a covalently linked FGFR2, FGFR3 and/or a SV2, such as, e.g., by chemical conjugation or by genetically produced fusion proteins. Methods that covalently link delivery agents and methods of using such agents are described in, e.g., Steven F. Dowdy, *Protein Transduction System and Methods of Use Thereof*, International Publication No WO 00/34308; Gérard Chassaing & Alain Prochiantz, *Peptides which can be Used as Vectors for the Intracellular Addressing of Active Molecules*, U.S. Patent No. 6,080,724 (Jun. 27, 2000); Alan Frankel et al., *Fusion Protein Comprising TAT-derived Transport Moiety*, U.S. Patent No. 5,674,980; Alan Frankel et al., *TAT-derived Transport Polypeptide Conjugates*, U.S. Patent No. 5,747,641; Alan Frankel et al., *TAT-derived Transport Polypeptides and Fusion Proteins*, U.S. Patent No. 5,804,604; Peter F. J. O'Hare et al., *Use of Transport Proteins*, U.S. Patent No. 6,734,167; Yao-Zhong Lin & Jack J. Hawiger, *Method for importing biologically active molecules into cells*, U.S. Patent No. 5,807,746; Yao-Zhong Lin & Jack J. Hawiger, *Method for importing biologically active molecules into cells*, U.S. Patent No. 6,043,339; Yao-Zhong Lin et al., *Sequence and Method for Genetic Engineering of Proteins with Cell Membrane Translocating Activity*, U.S. Patent No. 6,248,558; Yao-Zhong Lin et al., *Sequence and Method for Genetic Engineering of Proteins with Cell Membrane Translocating Activity*, U.S. Patent No. 6,432,680; Jack J. Hawiger et al., *Method for importing biologically active molecules into cells*, U.S. Patent No. 6,495,518; Yao-Zhong Lin et al., *Sequence and Method for Genetic Engineering of Proteins with Cell Membrane Translocating Activity*, U.S. Patent No. 6,780,843; Jonathan B. Rothbard & Paul A Wender, *Method and Composition for Enhancing Transport Across Biological Membranes*, U.S. Patent No. 6,306,993; Jonathan B. Rothbard & Paul A Wender, *Method and Composition for Enhancing Transport Across Biological Membranes*, U.S. Patent No. 6,495,663; and Pamela B. Davis et al., *Fusion proteins for protein delivery*, U.S. Patent No. 6,287,817.

[072] A delivery agent useful in the invention also can be an agent that enables or enhances cellular uptake of a non-covalently associated FGFR2, FGFR3 and/or a SV2. Methods that function in the absence of covalent linkage and methods of using such agents are described in, *e.g.*, Gilles Divita et al, *Peptide-mediated Transfection Agents and Methods of Use*, U.S. Patent No. 6,841,535; Philip L Felgner and Olivier Zelphati, *Intracellular Protein Delivery Compositions and Methods of Use*, U.S. Patent Publication No. 2003/0008813; and Michael Karas, *Intracellular Delivery of Small Molecules, Proteins and Nucleic Acids*, U.S. Patent Publication 2004/0209797. Such peptide delivery agents can be prepared and used by standard methods and are commercially available, see, *e.g.* the CHARIOT™ Reagent (Active Motif, Carlsbad, CA); BIOPORTER® Reagent (Gene Therapy Systems, Inc., San Diego, CA), BIOTREK™ Protein Delivery Reagent (Stratagene, La Jolla, CA), and PRO-JECT™ Protein Transfection Reagent (Pierce Biotechnology Inc., Rockford, IL).

[073] As mentioned above, a cell can stably contain a FGFR2, a FGFR3, a SV2, a FGFR2 and a SV2, a FGFR3 and a SV2, or a FGFR2, a FGFR3 and a SV2 disclosed in the present specification. Methods useful for making and using a cells that stably contain a FGFR2, a FGFR3, a SV2, or any combination thereof are described in, *e.g.*, Elizabeth E. Plowright et al., *Ectopic Expression of Fibroblast Growth Factor Receptor 3 Promotes Myeloma Cell Proliferation and Prevents Apoptosis*, 95(3) Blood 992-998 (2000); TC, see, *e.g.*, Hiroyuki Onose et al., *Over-Expression of Fibroblast Growth Factor Receptor 3 in a Human Thyroid Carcinoma Cell Line Results in Overgrowth of the Confluent Cultures*, 140(2) Eur. J. Endocrinol. 169-173 (1999); M. Kana et al., *Signal Transduction Pathway of Human Fibroblast Growth Factor Receptor 3. Identification of a Novel 66-kDa Phosphoprotein*, 272(10) J. Biol. Chem. 6621-6628 (1997); and Janet E. Henderson et al., *Expression of FGFR3 with the G380R Achondroplasia Mutation Inhibits Proliferation And Maturation of CFK2 Chondrocytic Cells*, 15(1) J. Bone Miner. Res. 155-165 (2000).

[074] Another aspect of the present invention provides, in part, an expression construct that allow for expression of a nucleic acid molecule encoding a FGFR2, a FGFR3 and/or a SV2 disclosed in the present specification. These expression constructs comprise an open reading frame encoding a FGFR2, a FGFR3 and/or a SV2 disclosed in the present specification, operably-linked to control sequences from an expression vector useful for expressing a FGFR2, a FGFR3 and/or a SV2 in a cell. The term "operably linked" as used herein, refers to any of a variety of cloning methods that can ligate a nucleic acid molecule disclosed in the present specification into an expression vector such that a peptide encoded by the composition is expressed when introduced into a cell. Well-established molecular biology techniques that may be necessary to make an expression construct disclosed in the present specification including, but not limited to, procedures involving polymerase chain reaction (PCR) amplification restriction enzyme reactions, agarose gel electrophoresis, nucleic acid ligation, bacterial transformation, nucleic acid purification, nucleic acid sequencing are routine procedures well within the scope of one skilled in the art and from the teaching herein. Non-limiting examples of specific protocols necessary to make an expression construct are described in *e.g.*, MOLECULAR CLONING A LABORATORY

MANUAL, *supra*, (2001); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Frederick M. Ausubel et al., eds. John Wiley & Sons, 2004). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[075] A wide variety of expression vectors can be employed for expressing an open reading frame encoding a FGFR2, a FGFR3 and/or a SV2 and include without limitation, viral expression vectors, prokaryotic expression vectors and eukaryotic expression vectors including yeast, insect and mammalian expression vectors. Non-limiting examples of expression vectors, along with well-established reagents and conditions for making and using an expression construct from such expression vectors are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; EMD Biosciences-Novagen, Madison, WI; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. The selection, making and use of an appropriate expression vector are routine procedures well within the scope of one skilled in the art and from the teachings herein.

[076] It is envisioned that any of a variety of expression systems may be useful for expressing construct compositions disclosed in the present specification. An expression system encompasses both cell-based systems and cell-free expression systems. Cell-based systems include, without limited, viral expression systems, prokaryotic expression systems, yeast expression systems, baculoviral expression systems, insect expression systems and mammalian expression systems. Cell-free systems include, without limitation, wheat germ extracts, rabbit reticulocyte extracts and *E. coli* extracts. Expression using an expression system can include any of a variety of characteristics including, without limitation, inducible expression, non-inducible expression, constitutive expression, viral-mediated expression, stably-integrated expression, and transient expression. Expression systems that include well-characterized vectors, reagents, conditions and cells are well-established and are readily available from commercial vendors that include, without limitation, Ambion, Inc. Austin, TX; BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; Roche Applied Science, Indianapolis, IN; and Stratagene, La Jolla, CA. Non-limiting examples on the selection and use of appropriate heterologous expression systems are described in *e.g.*, PROTEIN EXPRESSION. A PRACTICAL APPROACH (S. J. Higgins and B. David Hames eds., Oxford University Press, 1999); Joseph M. Fernandez & James P. Hoeffler, GENE EXPRESSION SYSTEMS. USING NATURE FOR THE ART OF EXPRESSION (Academic Press, 1999); and Meena Rai & Harish Padh, *Expression Systems for Production of Heterologous Proteins*, 80(9) CURRENT SCIENCE 1121-1128, (2001). These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[077] An expression construct comprising a nucleic acid molecule encoding a FGFR2, a FGFR3 and/or a SV2 disclosed in the present specification can be operationally-linked to a variety of regulatory elements that can positively or negatively modulate, either directly or indirectly, the expression of a nucleic acid molecule, such as, *e.g.*, constitutive, tissue-specific, inducible or synthetic promoters and enhancers. Non-limiting examples of constitutive regulatory elements include, *e.g.*, the cytomegalovirus

(CMV), herpes simplex virus thymidine kinase (HSV TK), simian virus 40 (SV40) early, 5' long terminal repeat (LTR), elongation factor-1 α (EF-1 α) and polyubiquitin (UbC) regulatory elements. Non-limiting examples of inducible regulatory elements useful in aspects of the present invention include, *e.g.*, chemical-inducible regulatory elements such as, without limitation, alcohol-regulated, tetracycline-regulated, steroid-regulated, metal-regulated and pathogenesis-related; and physical-inducible regulatory elements such as, without limitation, temperature-regulated and light-regulated. Such inducible regulatory elements can be prepared and used by standard methods and are commercially available, including, without limitation, tetracycline-inducible and tetracycline-repressible elements such as, *e.g.*, TET-ON[™] and TET-OFF[™] (BD Biosciences-Clontech, Palo Alto, CA) and the T-REX[™] (Tetracycline-Regulated Expression) and FLP-IN[™] T-REX[™] systems (Invitrogen, Inc., Carlsbad, CA); ecdysone-inducible regulatory elements such as, *e.g.*, the COMPLETE[®] Control Inducible Mammalian Expression System (Stratagene, Inc., La Jolla, CA); isopropyl β -D-galactopyranoside (IPTG)-inducible regulatory elements such as, *e.g.*, the LACSWITCH[®] II Inducible Mammalian Expression System (Stratagene, Inc., La Jolla, CA); and steroid-inducible regulatory elements such as, *e.g.*, the chimeric progesterone receptor inducible system, GENESWITCH[™] (Invitrogen, Inc., Carlsbad, CA). The skilled person understands that these and a variety of other constitutive and inducible regulatory systems are commercially available or well known in the art and can be useful in the invention for controlling expression of a nucleic acid molecule which encodes a BoNT/A receptor.

[078] In an embodiment, a nucleic acid molecule encoding a FGFR2 can optionally be linked to a regulatory element such as a constitutive regulatory element. In aspects of this embodiment, a nucleic acid molecule encoding a mammalian FGFR2 can optionally be linked to a regulatory element such as a constitutive regulatory element; a nucleic acid molecule encoding a bird FGFR2 can optionally be linked to a regulatory element such as a constitutive regulatory element; a nucleic acid molecule encoding an amphibian FGFR2 can optionally be linked to a regulatory element such as a constitutive regulatory element; and a nucleic acid molecule encoding a fish FGFR2 can optionally be linked to a regulatory element such as a constitutive regulatory element.

[079] In another embodiment, a nucleic acid molecule encoding a FGFR2 can optionally be linked to a regulatory element such as an inducible regulatory element. In aspects of this embodiment, a nucleic acid molecule encoding a mammalian FGFR2 can optionally be linked to a regulatory element such as a inducible regulatory element; a nucleic acid molecule encoding a bird FGFR2 can optionally be linked to a regulatory element such as a inducible regulatory element; a nucleic acid molecule encoding an amphibian FGFR2 can optionally be linked to a regulatory element such as a inducible regulatory element; and a nucleic acid molecule encoding a fish FGFR2 can optionally be linked to a regulatory element such as a inducible regulatory element. In another aspect of this embodiment, expression of the nucleic acid molecule is induced using, *e.g.*, tetracycline-inducible, ecdysone-inducible or steroid-inducible.

[080] In yet another embodiment, a nucleic acid molecule encoding a FGFR3 can optionally be linked to a regulatory element such as a constitutive regulatory element. In aspects of this embodiment, a nucleic acid molecule encoding a mammalian FGFR3 can optionally be linked to a regulatory element such as a constitutive regulatory element; a nucleic acid molecule encoding a bird FGFR3 can optionally be linked to a regulatory element such as a constitutive regulatory element; a nucleic acid molecule encoding an amphibian FGFR3 can optionally be linked to a regulatory element such as a constitutive regulatory element; and a nucleic acid molecule encoding a fish FGFR3 can optionally be linked to a regulatory element such as a constitutive regulatory element.

[081] In yet another embodiment, a nucleic acid molecule encoding a FGFR3 can optionally be linked to a regulatory element such as an inducible regulatory element. In aspects of this embodiment, a nucleic acid molecule encoding a mammalian FGFR3 can optionally be linked to a regulatory element such as a inducible regulatory element; a nucleic acid molecule encoding a bird FGFR3 can optionally be linked to a regulatory element such as a inducible regulatory element; a nucleic acid molecule encoding an amphibian FGFR3 can optionally be linked to a regulatory element such as a inducible regulatory element; and a nucleic acid molecule encoding a fish FGFR3 can optionally be linked to a regulatory element such as a inducible regulatory element. In another aspect of this embodiment, expression of the nucleic acid molecule is induced using, *e.g.*, tetracycline-inducible, ecdysone-inducible or steroid-inducible.

[082] In yet another embodiment, a nucleic acid molecule encoding a SV2 can optionally be linked to a regulatory element such as a constitutive regulatory element. In aspects of this embodiment, a nucleic acid molecule encoding a mammalian SV2 can optionally be linked to a regulatory element such as a constitutive regulatory element; a nucleic acid molecule encoding a bird SV2 can optionally be linked to a regulatory element such as a constitutive regulatory element; a nucleic acid molecule encoding an amphibian SV2 can optionally be linked to a regulatory element such as a constitutive regulatory element; and a nucleic acid molecule encoding a fish SV2 can optionally be linked to a regulatory element such as a constitutive regulatory element.

[083] In yet another embodiment, a nucleic acid molecule encoding a SV2 can optionally be linked to a regulatory element such as an inducible regulatory element. In aspects of this embodiment, a nucleic acid molecule encoding a mammalian SV2 can optionally be linked to a regulatory element such as a inducible regulatory element; a nucleic acid molecule encoding a bird SV2 can optionally be linked to a regulatory element such as a inducible regulatory element; a nucleic acid molecule encoding an amphibian SV2 can optionally be linked to a regulatory element such as a inducible regulatory element; and a nucleic acid molecule encoding a fish SV2 can optionally be linked to a regulatory element such as a inducible regulatory element. In another aspect of this embodiment, expression of the nucleic acid molecule is induced using, *e.g.*, tetracycline-inducible, ecdysone-inducible or steroid-inducible.

[084] It is understood that a FGFR2, a FGFR3 and/or a SV2 useful in aspects of the present invention optionally can include one or more additional components. As a non-limiting example, a flexible spacer sequence such as poly-glycine sequences can be included in a FGFR2, a FGFR3 and/or a SV2 useful in the invention. A useful FGFR2, FGFR3 and/or a SV2 can further include, without limitation, one or more of the following: epitope-binding tags, such as. e.g., FLAG, EXPRESS™, human Influenza virus hemagglutinin (HA), human p62^{c-Myc} protein (c-MYC), Vesicular Stomatitis Virus Glycoprotein (VSV-G), glycoprotein-D precursor of Herpes simplex virus (HSV), V5, and AU1; affinity-binding, such as. e.g., polyhistidine (HIS), streptavidin binding peptide (strep), and biotin or a biotinylation sequence; peptide-binding regions, such as. e.g., the glutathione binding domain of glutathione-S-transferase, the calmodulin binding domain of the calmodulin binding protein, and the maltose binding domain of the maltose binding protein; immunoglobulin hinge region; an N-hydroxysuccinimide linker; a peptide or peptidomimetic hairpin turn; or a hydrophilic sequence or another component or sequence that, for example, promotes the solubility or stability of a FGFR2, a FGFR3, and/or a SV2. Non-limiting examples of specific protocols for selecting, making and using an appropriate binding peptide are described in, e.g., *Epitope Tagging*, pp. 17.90-17.93 (Sambrook and Russell, eds., MOLECULAR CLONING A LABORATORY MANUAL, Vol. 3, 3rd ed. 2001); ANTIBODIES: A LABORATORY MANUAL (Edward Harlow & David Lane, eds., Cold Spring Harbor Laboratory Press, 2nd ed. 1998); and USING ANTIBODIES: A LABORATORY MANUAL: PORTABLE PROTOCOL NO. I (Edward Harlow & David Lane, Cold Spring Harbor Laboratory Press, 1998). In addition, non-limiting examples of binding peptides as well as well-characterized reagents, conditions and protocols are readily available from commercial vendors that include, without limitation, BD Biosciences-Clontech, Palo Alto, CA; BD Biosciences Pharmingen, San Diego, CA; Invitrogen, Inc, Carlsbad, CA; QIAGEN, Inc., Valencia, CA; and Stratagene, La Jolla, CA. These protocols are routine procedures well within the scope of one skilled in the art and from the teaching herein.

[085] Aspects of the present invention provide, in part, a cell that contains an exogenous FGFR2 wherein said cell is capable of being intoxicated by a BoNT/A; a cell that contains an exogenous FGFR3 wherein said cell is capable of being intoxicated by a BoNT/A; or a cell that contains an exogenous SV2 wherein said cell is capable of BoNT/A intoxication; or a cell that contains any combination of an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, wherein said cell is capable of BoNT/A intoxication. As used herein, the term "cell," means any eukaryotic cell that expresses at least one endogenous FGFR2 or at least one endogenous FGFR3, or can be engineered to express, at least one exogenous FGFR2 that binds BoNT/A or at least one exogenous FGFR3 that binds BoNT/A. Such cells can optionally express at least one endogenous SV2, or can be engineered to express, at least one exogenous SV2. The term cell encompasses cells from a variety of organisms, such as, e.g., murine, rat, porcine, bovine, equine, primate and human cells; from a variety of cell types such as, e.g., neural and non-neural; and can be isolated from or part of a heterogeneous cell population, tissue or organism. It is understood that cells useful in aspects of the invention can included, without limitation, primary cells; cultured cells; established cells; normal cells; transformed cells; tumor cells; infected cells; proliferating and terminally differentiated cells; and stably or transiently transfected cells, including stably and transiently transfected cells. It is further understood that cells useful in aspects of the invention can be in

any state such as proliferating or quiescent; intact or permeabilized such as through chemical-mediated transfection such as, *e.g.*, calcium phosphate-mediated, diethy-laminoethyl (DEAE) dextran-mediated, lipid-mediated, polyethyleneimine (PEI)-mediated, polybrene-mediated, and protein delivery agents; physical-mediated transfection, such as, *e.g.*, biolistic particle delivery, microinjection and electroporation; and viral-mediated transfection, such as, *e.g.*, retroviral-mediated transfection. It is further understood that cells useful in aspects of the invention may include those which express a FGFR2, a FGFR3 or a SV2 under control of a constitutive, tissue-specific, cell-specific or inducible promoter element, enhancer element or both.

[086] As used herein, the term “cell capable of BoNT/A intoxication” means a cell that can enable the overall cellular mechanism whereby BoNT/A proteolytically cleaves a substrate, such as, *e.g.*, SNAP-25, and encompasses the binding of BoNT/A to a low or high affinity receptor and/or a receptor complex, the internalization of the toxin/receptor complex, the translocation of the BoNT/A light chain into the cytoplasm and the enzymatic target modification of a BoNT/A substrate. By definition, a cell capable of BoNT/A intoxication must express a FGFR2 or a FGFR3. As a non-limiting example, a neuronal or non-neuronal cell can be transiently or stably engineered to express an exogenous nucleic acid molecule encoding a FGFR2 and/or a FGFR3. As another non-limiting example, a neuronal or non-neuronal cell can be transiently engineered to contain an exogenous FGFR2 and/or an exogenous FGFR3. As yet another non-limiting example, a neuronal or non-neuronal cell can be transiently or stably engineered to express an exogenous nucleic acid molecule encoding a SV2. As yet another non-limiting example, a neuronal or non-neuronal cell can be transiently engineered to contain an exogenous SV2. Other examples of useful cells include, without limitation, a neuronal or non-neuronal cell comprising an endogenous FGFR2 and/or an endogenous FGFR3 that is transiently or stably engineered to express an exogenous nucleic acid molecule encoding a SV2; a neuronal or non-neuronal cell comprising an endogenous FGFR2 and/or an endogenous FGFR3 that is transiently or stably engineered to express an exogenous SV2; a neuronal or non-neuronal cell comprising an endogenous SV2 that is transiently or stably engineered to express an exogenous nucleic acid molecule encoding a FGFR2 and/or a FGFR3; a neuronal or non-neuronal cell comprising an endogenous SV2 that is transiently or stably engineered to express an exogenous FGFR2 and/or an exogenous FGFR3; a neuronal or non-neuronal cell comprising an endogenous FGFR2, an endogenous FGFR3 and an endogenous SV2; and a neuronal or non-neuronal cell that is transiently or stably engineered to express an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2. In all the above combinations, a neuronal or non-neuronal cell can further comprise a ganglioside.

[087] Cells useful in aspects of the invention include both neuronal and non-neuronal cells. Neuronal cells useful in aspects of the invention include, without limitation, primary neuronal cells; immortalized or established neuronal cells; transformed neuronal cells; neuronal tumor cells; stably and transiently transfected neuronal cells and further include, yet are not limited to, mammalian, murine, rat, primate and human neuronal cells. Non-limiting examples of neuronal cells useful in aspects of the invention include, *e.g.*, peripheral neuronal cells, such as, *e.g.*, motor neurons and sensory neurons; and CNS neuronal

cells, such as, e.g., spinal cord neurons like embryonic spinal cord neurons, dorsal root ganglia (DRG) neurons, cerebral cortex neurons, cerebellar neurons, hippocampal neurons and motor neurons. Neuronal cells useful in the invention can be, for example, central nervous system (CNS) neurons; neuroblastoma cells; motor neurons, hippocampal neurons or cerebellar neurons and further can be, without limitation, Neuro-2A, SH-SY5Y, NG108-15, N1E-115, SiMa, PC-12, or SK-N-DZ cells. The skilled person understands that these and additional primary and established neurons can be useful in the cells and methods of the invention.

[088] Neurons useful in aspects of the invention include, without limitation, primary cultures such as primary cultures of embryonic dorsal root ganglion (DRG) neurons. As one example, primary cultures of embryonic rat DRG neurons are described in Mary J. Welch et al., *Sensitivity of Embryonic Rat Dorsal Root Ganglia Neurons to Clostridium Botulinum Neurotoxins*, 38(2) *Toxicon* 245-258 (2000); and primary cultures of fetal spinal cord neurons, for example, primary cultures of murine fetal spinal cord neurons are described in Elaine A. Neale et al., *Botulinum Neurotoxin A Blocks Synaptic Vesicle Exocytosis but not Endocytosis at the Nerve Terminal*, 147(6) *J. Cell Biol.* 1249-1260 (1999), and John A. Chaddock et al., *Inhibition of Vesicular Secretion in Both Neuronal and Non-Neuronal Cells by a Retargeted Endopeptidase Derivative of Clostridium Botulinum Neurotoxin Type A*, 68(5) *Infect. Immun.* 2587-2593 (2000). Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a neuron that contains an exogenous FGFR3. In aspects of this embodiment, a neuron can be a neuron from, e.g., a primary culture, an embryonic dorsal root ganglion primary culture or a fetal spinal cord primary culture. As non-limiting examples, cells useful according to a method disclosed in the present specification can include, a primary neuronal cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, such as, e.g., a rat embryonic dorsal root ganglion (DRG) neuron that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[089] Neuronal cell lines useful in aspects of the invention include, without limitation, neuroblastoma cell lines, neuronal hybrid cell lines, spinal cord cell lines, central nervous system cell lines, cerebral cortex cell lines, dorsal root ganglion cell lines, hippocampal cell lines and pheochromocytoma cell lines.

[090] Neuroblastoma cell lines, such as, e.g., murine, rat, primate or human neuroblastoma cell lines can be useful in aspects of the invention. Neuroblastoma cell lines useful in aspects of the invention include, without limitation, BE(2)-C (ATCC CRL-2268; ECACC 95011817), BE(2)-M17 (ATCC CRL-2267; ECACC 95011816), C1300 (ECACC 93120817), CHP-212 (ATCC CRL-2273), CHP-126 (DSMZ ACC 304), IMR 32 (ATCC CRL-127; ECACC 86041809; DSMZ ACC 165), KELLY (ECACC 92110411; DSMZ ACC 355), LA-N-2, see, e.g., Robert C. Seeger et al., *Morphology, Growth, Chromosomal Pattern and Fibrinolytic Activity of Two New Human Neuroblastoma Cell Lines*, 37(5) *Cancer Res.* 1364-1371 (1977); and G. J. West et al., *Adrenergic, Cholinergic, And Inactive Human Neuroblastoma Cell Lines With the Action-Potential Na⁺ Ionophore*, 37(5) *Cancer Res.* 1372-1376 (1977), MC-IXC (ATCC CRL-2270), MHH-

NB-11 (DSMZ ACC 157), N18Tg2 (DSMZ ACC 103), N1E-115 (ATCC CCL-2263; ECACC 88112303), N4TG3 (DSMZ ACC 101), Neuro-2A (ATCC CCL-131; ECACC 89121404; DSMZ ACC 148), NB41A3 (ATCC CCL-147; ECACC 89121405), NS20Y (DSMZ ACC 94), SH-SY5Y (ATCC CRL-2266; ECACC 94030304; DSMZ ACC 209), SiMa (DSMZ ACC 164), SK-N-DZ (ATCC CRL-2149; ECACC 94092305), SK-N-F1 (ATCC CRL-2142, ECACC 94092304), SK-N-MC (ATCC HTB-10, DSMZ ACC 203) and SK-N-SH (ATCC HTB-11, ECACC 86012802). Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a neuroblastoma cell that contains an exogenous FGFR3, an exogenous FGFR2, an exogenous SV2, or an exogenous FGFR3 and an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous FGFR2, or an exogenous FGFR3 an exogenous FGFR2 and an exogenous SV2. In aspects of this embodiment, a neuroblastoma cell can be, e.g., BE(2)-C, BE(2)-M17, C1300, CHP-212, CHP-126, IMR 32, KELLY, LA-N-2, MC-IXC, MHH-NB-11, N18Tg2, N1E-115, N4TG3, Neuro-2A, NB41A3, NS20Y, SH-SY5Y, SiMa, SK-N-DZ, SK-N-F1, SK-N-MC and SK-N-SH. As non-limiting examples, cells useful for detecting BoNT/A activity according to a method disclosed in the present specification can include, a neuroblastoma cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, such as, e.g., a SH-SY5Y cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof; a Neuro-2a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof; and a N1E-115 cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof; and a SK-N-DZ cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[091] Neuronal hybrid cell lines, such as, e.g., murine, rat, primate and human hybrid neuronal cell lines can be useful in aspects of the invention. Such hybrid cell lines include neuroblastoma/glioma hybrids, such as, e.g., N18 (ECACC 88112301), NG108-15 (ATCC HB-12317, ECACC 88112302) and NG115-401L (ECACC 87032003); neuroblastoma/motor neuron hybrids, such as, e.g., NSC-19 and NSC-34, which express motor neuron characteristics, display a multipolar neuron-like phenotype, express high levels of choline acetyltransferase (CHAT), generate action potentials, express neurofilament triplet proteins and synthesize, store and release acetylcholine., see, e.g., N. R. Cashman et al., *Neuroblastoma X Spinal Cord (NSC) Hybrid Cell Lines Resemble Developing Motor Neurons*, 194(3) Dev. Dyn. 209-221 (1992); and Christopher J. Eggett et al., *Development and Characterization of a Glutamate-Sensitive Motor Neuronal Cell Line*, 74(5) J. Neurochem. 1895-1902 (2000); neuroblastoma/root ganglion neuron hybrids, such as, e.g., F11, see, e.g., Doros Platika et al., *Neuronal Traits of Clonal Cell Lines Derived by Fusion of Dorsal Root Ganglia Neurons With Neuroblastoma Cells*, 82(10) Proc. Natl. Acad. Sci. U. S. A. 3499-3503 (1985), ND-E (ECACC 92090915), ND-U1 (ECACC 92090916), ND7/23 (ECACC 92090903), ND8/34 (ECACC 92090904) and ND27 (ECACC 92090912); neuroblastoma/hippocampal neuron hybrids, such as, e.g., HN-33, see, e.g., Henry J. Lee et al., *Neuronal Properties and Trophic Activities of Immortalized Hippocampal Cells From Embryonic and Young Adult Mice*, 10(6) J. Neurosci. 1779-1787 (1990). Thus, in an embodiment, a cell capable of

BoNT/A toxin intoxication can be a hybrid neuron that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2. In aspects of this embodiment, a hybrid neuron can be, e.g., a neuroblastoma/glioma hybrid cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, a neuroblastoma/motor neuron hybrid cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, a neuroblastoma/root ganglion neuron hybrid cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof and a neuroblastoma/ hippocampal neuron hybrid cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof. In further aspects of this embodiment, a neuroblastoma/glioma hybrid can be, e.g., N18, NG108-15 and NG115-401L. In further aspects of this embodiment, a neuroblastoma/motor neuron hybrid can be, e.g., NSC-19 and NSC-32. In further aspects of this embodiment, a neuroblastoma/root ganglion neuron hybrid can be, e.g., F11, ND-E, ND-U1, ND7/23, ND8/34 and ND27. In further aspects of this embodiment, a neuroblastoma/hippocampal neuron hybrid can be, e.g., HN-33. As non-limiting examples, cells useful for detecting BoNT/A activity according to a method disclosed in the present specification can include, a neuronal hybrid cell, such as, e.g., a NG108-15 cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[092] Spinal cord cell lines, such as, e.g., murine, rat, primate or human spinal cord cell lines can be useful in aspects of the invention and include, without limitation, TE 189.T (ATCC CRL-7947) and M4b, see, e.g., Ana M. Cardenas et al., *Establishment and Characterization of Immortalized Neuronal Cell Lines Derived From the Spinal Cord of Normal and Trisomy 16 Fetal Mice, an Animal Model of Down syndrome*, 68(1) J. Neurosci. Res. 46-58 (2002). As an example, a human spinal cord cell line can be generated from precursors of human embryonic spinal cord cells (first trimester embryos) that are immortalized with a tetracycline repressible v-myc oncogene as described in Ronghao Li et al., *Motoneuron Differentiation of Immortalized Human Spinal Cord Cell Lines*, 59(3) J. Neurosci. Res. 342-352 (2000). Such cells can be expanded indefinitely in proliferative growth conditions before rapid differentiation (4-7 days) into functional neurons that express neuronal phenotypic markers such as choline acetyltransferase. As another example, a murine spinal cord cell line can be prepared by immortalizing an embryonic spinal cord culture using transforming media. Such a spinal cord cell line can be, for example, the murine M4b line and can express neuronal markers such as NSE, synaptophysin, MAP 2 and choline acetyltransferase, and can release acetylcholine upon appropriate stimulation, see, e.g., Cardenas et al., *supra*, (2002). Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a spinal cord cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2,. In aspects of this embodiment, a spinal cord cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof can be, e.g., a TE 189.T cell that contains an exogenous FGFR2, an exogenous

FGFR3, an exogenous SV2, or any combination thereof and a M4b cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[093] Central nervous system (CNS) cell lines, such as, e.g., murine, rat, primate and human CNS cell lines, can be useful in aspects of the invention. A useful CNS cell line can be, for example, a human CNS cell line immortalized with a tetracycline repressible v-myc oncogene as described in Dinah W. Sah et al., *Bipotent Progenitor Cell Lines From the Human CNS*, 15(6) Nat. Biotechnol. 574-580 (1997). Upon repression of the oncogene, the cells differentiate into neurons. Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a CNS cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2.

[094] Cerebral cortex cell lines, such as, e.g., murine, rat, primate and human cerebral cortex cell lines, can be useful in aspects of the invention and include, without limitation, CNh, see, e.g., Ana M. Cardenas et al., *Calcium Signals in Cell Lines Derived From the Cerebral Cortex of Normal and Trisomy 16 Mice*, 10(2) Neuroreport 363-369 (1999), HCN-1a (ATCC CRL-10442) and HCN-2 (ATCC CRL-10742). As an example, murine cortex primary cultures from 12-16 days embryos can be immortalized, for example, by culturing the cells in conditioned media from a rat thyroid cell line that induces transformation *in vitro*. The immortalized cells can be differentiated into neurons expressing neuronal markers using the appropriate media; these differentiated cells express choline acetyltransferase and secrete acetylcholine and glutamate in response to depolarization and nicotine stimulation, see, e.g., David D. Allen et al., *Impaired Cholinergic Function in Cell Lines Derived From the Cerebral Cortex Of Normal And Trisomy 16 Mice*, 12(9) Eur. J. Neurosci. 3259-3264 (2000). Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a cerebral cortex cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2. In aspects of this embodiment, a cerebral cortex cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof can be, e.g., a CNh cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, HCN-1a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof and HCN-2 cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[095] Dorsal root ganglia cell lines, such as, e.g., murine, rat, primate and human dorsal root ganglia cell lines, can be useful in aspects of the invention and include, without limitation, G4b, see, e.g., David D. Allen et al., *A Dorsal Root Ganglia Cell Line Derived From Trisomy 16 Fetal Mice, a Model for Down Syndrome*, 13(4) Neuroreport 491-496 (2002). Embryonic dorsal root ganglia primary cultures can be immortalized with transforming conditioned media as described above. Upon differentiation, the cell line exhibits neuronal traits and lacks glial markers by immunohistochemistry. Release of neurotransmitters such as acetylcholine can be induced in response to potassium and nicotine, see, e.g., Allen et al., *supra*,

(2002). Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a dorsal root ganglia cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2. In aspects of this embodiment, a dorsal root ganglia cell can be, e.g., a G4b cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[096] Hippocampal cell lines, such as, e.g., murine, rat, primate and human hippocampal lines can be useful in aspects of the invention and include, without limitation, HT-4, see, e.g., K. Frederiksen et al., *Immortalization of Precursor Cells From the Mammalian CNS*, 1(6) Neuron 439-448 (1988) and HT-22, see, e.g., John B. Davis and Pamela Maher, *Protein Kinase C Activation Inhibits Glutamate-Induced Cytotoxicity in a Neuronal Cell Line*, 652(1) Brain Res. 169-173 (1994). As a non-limiting example, the murine hippocampal cell line HT-22 can be useful in the invention. As a further non-limiting example, the immortalized HN33 hippocampal cell line can be useful in the invention. This hippocampal cell line was derived from the fusion of primary neurons from the hippocampus of postnatal day 21 mice with the N18TG2 neuroblastoma cell line, and, when differentiated, shares membrane properties with adult hippocampal neurons in primary culture, see, e.g., Henry J. Lee et al., *Neuronal Properties and Trophic Activities of Immortalized Hippocampal Cells from Embryonic and Young Adult Mice*, 19(6) J. Neurosci. 1779-1787 (1990); and Henry J. Lee et al., *Immortalized Young Adult Neurons From the Septal Region: Generation and Characterization*, 52(1-2) Brain Res. Dev Brain Res. 219-228 (1990). Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a hippocampal cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2. In aspects of this embodiment, a hippocampal cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof can be, e.g., a HT-4 cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, a HT-22 cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof and a HN33 cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[097] A variety of non-neuronal cells are useful in aspects of the invention. Non-neuronal cells useful in aspects of the invention include, without limitation, primary non-neuronal cells; immortalized or established non-neuronal cells; transformed non-neuronal cells; non-neuronal tumor cells; stably and transiently transfected non-neuronal cells and further include, yet are not limited to, mammalian, murine, rat, primate and human non-neuronal cells. Non-neuronal cells useful in aspects of the invention further include, without limitation, any of the following primary or established cells: anterior pituitary cells; adrenal cells, such as, e.g., chromaffin cells of the adrenal medulla; pancreatic cells, such as, e.g., pancreatic acinar cells, pancreatic islet β cells and insulinoma HIT or INS-1 cells; ovarian cells, such as, e.g., steroid-producing ovarian cells; kidney cells, such as, e.g., inner medullary collecting duct (IMCD) cells; stomach cells, such as, e.g., enterochromaffin cells; blood cells, such as, e.g., eurythrocytes, leucocytes,

platelets, neutrophils, eosinophils, mast cells; epithelial cells, such as, e.g., those of the apical plasma membrane; fibroblasts; thyroid cells; chondrocytes; muscle cells; hepatocytes; glandular cells such as, e.g., pituitary cells, adrenal cells, chromaffin cells; and cells involved in glucose transporter (GLUT4) translocation. Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a non-neuronal cell. In aspects of this embodiment, a non-neuronal cell can be from a primary or established non-neuronal cell line from the, e.g., anterior pituitary cells, adrenal cells, pancreatic cells, ovarian cells, kidney cells, stomach cells, blood cells, epithelial cells, fibroblasts, thyroid cells, chondrocytes, muscle cells, hepatocytes and glandular cells.

[098] As non-limiting examples, cells useful for detecting BoNT/A activity according to a method disclosed in the present specification can include, a primary or established non-neuronal cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2, such as, e.g., a chromaffin cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof or pancreatic acinar cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.

[099] As discussed above, cells useful in the invention include neuronal and non-neuronal cells that express low or undetectable levels of endogenous FGFR2, endogenous FGFR3, endogenous SV2, or any combination thereof, but which have been transfected with, or otherwise genetically engineered to express, one or more exogenous nucleic acid molecules encoding one or more FGFR2s, FGFR3s and/or SV2. Cells useful in aspects of the present invention further include, without limitation, transformed, tumor or other cells which over-express one or more exogenous FGFR2s, one or more exogenous FGFR3s and/or one or more exogenous SV2s. It is understood that the over-expressed receptor can be a wild type form of the receptor or can include one or more amino acid modifications as compared to the wild type receptor, with the proviso that the process of BoNT/A intoxication can still occur. As a non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous mammalian FGFR2, such as, e.g., a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous bird FGFR2, such as, e.g., chicken FGFR2. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous amphibian FGFR2, such as, e.g., a newt FGFR2 or a frog FGFR2. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous fish FGFR2, such as, e.g., a zebrafish FGFR2. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous mammalian FGFR3, such as, e.g., a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous bird FGFR3, such as, e.g., chicken FGFR3. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those

which express or over-express an exogenous amphibian FGFR3, such as, *e.g.*, a newt FGFR3 or a frog FGFR3. As another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous fish FGFR3, such as, *e.g.*, a zebrafish FGFR3. As yet another non-limiting example, cells useful for detecting BoNT/A activity encompass those which express or over-express an exogenous SV2, such as, *e.g.*, a SV2A, a SV2B, or a SV2C.

[0100] Thus, in an embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, an exogenous FGFR2 and an exogenous SV2, an exogenous FGFR3 and an exogenous SV2, or an exogenous FGFR2, an exogenous FGFR3 and an exogenous SV2. In aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous mammalian FGFR2, such as, *e.g.*, a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous bird FGFR2, such as, *e.g.*, chicken FGFR2. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous amphibian FGFR2, such as, *e.g.*, a newt FGFR2 or a frog FGFR2. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous fish FGFR2, such as, *e.g.*, a zebrafish FGFR2. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous mammalian FGFR3, such as, *e.g.*, a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous bird FGFR3, such as, *e.g.*, chicken FGFR3. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous amphibian FGFR3, such as, *e.g.*, a newt FGFR3 or a frog FGFR3. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous fish FGFR3, such as, *e.g.*, a zebrafish FGFR3. In other aspects of this embodiment, a cell capable of BoNT/A intoxication can be a cell stably expressing an exogenous SV2, such as, *e.g.*, a SV2A, a SV2B, or a SV2C.

[0101] Aspects of the present invention provide, in part, detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity. As used herein, the term "control cell" means a cell of the same or similar type as the contacted cell and grown under the same conditions but which is not contacted with any sample or is contacted with a defined negative sample or a defined positive sample. One skilled in the art understands that a variety of control cells are useful in the methods disclosed in the present specification and that a control cell can be a positive control cell or a negative control cell. A control cell can be, for example, a negative control cell such as a similar or identical cell containing the same or similar FGFR2, FGFR3 and/or a SV2 that is contacted with a similar, defined negative sample, which is known to lack active BoNT/A, or that is not contacted with any sample. A control cell also can be, for example, a positive control cell such as a similar or identical cell containing the same or similar FGFR2, FGFR3 and/or a SV2 contacted with a defined positive sample, which is known to include active BoNT/A.

[0102] A wide variety of assays can be used to determine the presence of BoNT/A activity, including direct and indirect assays for toxin uptake. Assays that determine BoNT/A binding or uptake properties can be used to assess BoNT/A activity. Such assays include, without limitation, cross-linking assays using labeled BoNT/A, such as, e.g., BoNT/A-SBED, see, e.g., Example II of the present specification and [¹²⁵I] BoNT/A, see, e.g., Noriko Yokosawa et al., *Binding of Clostridium botulinum Type C Neurotoxin to Different Neuroblastoma Cell Lines*, 57(1) Infect. Immun. 272-277 (1989); Noriko Yokosawa et al., *Binding of Botulinum Type C1, D and E Neurotoxins to Neuronal Cell Lines and Synaptosomes*, 29(2) Toxicon 261-264 (1991); and Tei-ichi Nishiki et al., *Identification of Protein Receptor for Clostridium Botulinum Type B Neurotoxin in Rat Brain Synaptosomes*, 269(14) J. Biol. Chem. 10498-10503 (1994). Other non-limiting assays include immunocytochemical assays that detect toxin binding using labeled or unlabeled antibodies, see, e.g., Atsushi Nishikawa et al., *The Receptor and Transporter for Internalization of Clostridium Botulinum Type C Progenitor Toxin into HT-29 Cells*, 319(2) Biochem. Biophys. Res. Commun. 327-333 (2004) and immunoprecipitation assays, see, e.g., Yukako Fujinaga et al., *Molecular Characterization of Binding Subcomponents of Clostridium Botulinum Type C Progenitor Toxin for Intestinal Epithelial Cells and Erythrocytes*, 150(Pt 5) Microbiology 1529-1538 (2004). Antibodies useful for these assays include, without limitation, antibodies selected against a BoNT/A, antibodies selected against a BoNT/A receptor, such as, e.g., FGFR3 or a SV2, antibodies selected against a ganglioside, such as, e.g., GD1a, GD1b, GD3, GQ1b, or GT1b and selected against a test compound, such as, e.g., a molecule that selectively binds a BoNT/A receptor wherein selective binding modulates BoNT/A activity. If the antibody is labeled, the binding of the molecule can be detected by various means, including Western blotting, direct microscopic observation of the cellular location of the antibody, measurement of cell or substrate-bound antibody following a wash step, or electrophoresis, employing techniques well-known to those of skill in the art. If the antibody is unlabeled, one may employ a labeled secondary antibody for indirect detection of the bound molecule, and detection can proceed as for a labeled antibody. It is understood that these and similar assays that determine BoNT/A uptake properties or characteristics can be useful in detecting BoNT/A activity.

[0103] Assays that monitor the release of a molecule after exposure to BoNT/A can also be used to assess for the presence of BoNT/A activity. In these assays, inhibition of the molecule's release would occur in cells expressing a FGFR2, a FGFR3 and/or a SV2 after BoNT/A treatment. As a non-limiting example the inhibition of insulin release assay disclosed in the present specification can monitor the release of a molecule after exposure to BoNT/A and thereby be useful in assessing whether a molecule selectively binds a BoNT/A receptor (see Example I). Other non-limiting assays include methods that measure inhibition of radio-labeled catecholamine release from neurons, such as, e.g., [³H] noradrenaline or [³H] dopamine release, see e.g., A Fassio et al., *Evidence for Calcium-Dependent Vesicular Transmitter Release Insensitive to Tetanus Toxin and Botulinum Toxin Type F*, 90(3) Neuroscience 893-902 (1999); and Sara Stigliani et al., *The Sensitivity of Catecholamine Release to Botulinum Toxin C1 and E Suggests Selective Targeting of Vesicles Set into the Readily Releasable Pool*, 85(2) J. Neurochem. 409-421 (2003), or measures catecholamine release using a fluorometric procedure, see,

e.g., Anton de Paiva et al., *A Role for the Interchain Disulfide or its Participating Thiols in the Internalization of Botulinum Neurotoxin A Revealed by a Toxin Derivative that Binds to Ecto-Acceptors and Inhibits Transmitter Release Intracellularly*, 268(28) J. Biol. Chem. 20838-20844 (1993); Gary W. Lawrence et al., *Distinct Exocytotic Responses of Intact and Permeabilised Chromaffin Cells After Cleavage of the 25-Kda Synaptosomal-Associated Protein (SNAP-25) or Synaptobrevin by Botulinum Toxin A or B*, 236(3) Eur. J. Biochem. 877-886 (1996); and Patrick Foran et al., *Botulinum Neurotoxin C1 Cleaves Both Syntaxin and SNAP-25 in intact and Permeabilized Chromaffin Cells: Correlation With its Blockade of Catecholamine Release*, 35(8) Biochemistry 2630-2636 (1996); and methods that measure inhibition of hormone release from endocrine cells, such as, e.g., anterior pituitary cells or ovarian cells. It is understood that these and similar assays for molecule release can be useful in assessing BoNT/A activity.

[0104] As non-limiting examples, an inhibition of insulin release assay can be used to determine the presence of BoNT/A activity in cells containing a FGFR2, a FGFR3 and/or a SV2 and capable of secreting insulin; an inhibition of noradrenaline release assay can be used to determine BoNT/A activity in cells containing a FGFR2, a FGFR3 and/or a SV2 and capable of secreting noradrenaline; and an inhibition of estrogen release assay can be used to determine BoNT/A activity in cells containing a FGFR2, a FGFR3 and/or a SV2 and capable of secreting estrogen.

[0105] Assays that detect the cleavage of a BoNT/A substrate after exposure to BoNT/A can also be used to assess for the presence of BoNT/A activity. In these assays, generation of a BoNT/A cleavage-product would be detected after BoNT/A treatment. As a non-limiting example the SNAP-25 cleavage assay disclosed in the present specification can detect the cleavage of a BoNT/A substrate after exposure to BoNT/A and thereby be useful in assessing BoNT/A activity (see Example I). Other non-limiting methods useful to detect the cleavage of a BoNT/A substrate after exposure to BoNT/A are described in, e.g., Lance E. Steward et al., *FRET Protease Assays for Botulinum Serotype A/E Toxins*, U.S. Patent 7,332,567; Lance E. Steward et al., *FRET Protease Assays for Clostridial Toxins*, U.S. Patent 7,208,285; Ester Fernandez-Salas et al., *Cell-based Fluorescence Resonance Energy Transfer (FRET) Assays for Clostridial Toxins*, U.S. Patent 7,183,006; and Ester Fernandez-Salas et al., *Immuno-Based Botulinum Toxin Serotype A Activity Assays*, U.S. Patent Application 12/403,531, each of which is hereby incorporated by reference in its entirety. It is understood that these and similar assays for BoNT/A substrate cleavage can be useful in assessing BoNT/A activity.

[0106] As non-limiting examples, Western blot analysis using an antibody that recognizes BoNT/A SNAP-25-cleaved product can be used to determine the presence of BoNT/A activity. Examples of anti-SNAP-25 antibodies useful for these assays include, without limitation, rabbit polyclonal anti-SNAP25₁₉₇ antiserum pAb anti-SNAP-25₁₉₇ #1 (Allergan, Inc., Irvine, CA), mouse monoclonal anti-SNAP-25 antibody SMI-81 (Sternberger Monoclonals, Lutherville, MD), mouse monoclonal anti-SNAP-25 antibody CI 71.1 (Synaptic Systems, Goettingen, Germany), mouse monoclonal anti-SNAP-25 antibody CI 71.2 (Synaptic Systems, Goettingen, Germany), mouse monoclonal anti-SNAP-25 antibody SP12 (Abcam, Cambridge,

MA), rabbit polyclonal anti-SNAP-25 antiserum (Synaptic Systems, Goettingen, Germany), and rabbit polyclonal anti-SNAP-25 antiserum (Abcam, Cambridge, MA).

[0107] The methods disclosed in the present specification include, in part, a sample. As used herein, the term “sample” means any biological matter that contains or potentially contains an active BoNT/A. A variety of samples can be assayed according to a method disclosed in the present specification including, without limitation, purified, partially purified, or unpurified BoNT/A; recombinant single chain or di-chain toxin with a naturally or non-naturally occurring sequence; recombinant BoNT/A with a modified protease specificity; recombinant BoNT/A with an altered cell specificity; chimeric toxin containing structural elements from multiple BoNT/A species or subtypes; bulk BoNT/A; formulated BoNT/A product; and foods; cells or crude, fractionated or partially purified cell lysates, for example, engineered to include a recombinant nucleic acid encoding a BoNT/A; bacterial, baculoviral and yeast lysates; raw, cooked, partially cooked or processed foods; beverages; animal feed; soil samples; water samples; pond sediments; lotions; cosmetics; and clinical formulations. It is understood that the term sample encompasses tissue samples, including, without limitation, mammalian tissue samples; livestock tissue samples such as sheep, cow and pig tissue samples; primate tissue samples; and human tissue samples. Such samples encompass, without limitation, intestinal samples such as infant intestinal samples, tissue samples obtained from a wound. Other such samples include mammalian tissue, mammalian saliva, mammalian excretions and mammalian feces. As non-limiting examples, a method of the invention can be useful for detecting the presence or activity of a BoNT/A in a food or beverage sample; to assay a sample from a human or animal, for example, exposed to a BoNT/A or having one or more symptoms of a BoNT/A exposure; to follow activity during production and purification of BoNT/A; or to assay formulated BoNT/A products such as pharmaceuticals or cosmetics.

[0108] It is envisioned that a wide variety of processing formats can be used in conjunction with the methods disclosed present specification, including, without limitation, manual processing, partial automated-processing, semi-automated-processing, full automated-processing, high throughput processing, high content processing, and the like or any combination thereof.

[0109] Other aspect of the present invention provide methods of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a FGFR2 and/or a FGFR3 wherein said selective binding reduces the ability of BoNT/A to bind to said FGFR2 and/or said FGFR3. It is envisioned that any molecule that can selectively bind to a FGFR2 and/or a FGFR3 in a manner that prevents BoNT/A binding to that same FGFR2 and/or FGFR3 can be useful, including, without limitation, an anti-FGFR2 antibody, an anti-FGFR3 antibody, an FGF antagonist or an FGF agonist. In addition, a FGFR2 and/or a FGFR3, a FGFR2 and/or a FGFR3 fragment retaining BoNT/A selective binding activity, or peptidomimetic thereof can also be useful. Molecules that selectively bind a FGFR2 and/or a FGFR3, and thus useful in methods of reducing BoNT/A activity are described in, e.g., Avner Yayon et al., *Antibodies that Block Receptor Protein Tyrosine Kinase Activation, Methods of Screening for and Using Thereof*, International Publication No.

WO 02/102972; Avner Yayon et al., *Antibodies that Block Receptor Protein Tyrosine Kinase Activation, Methods of Screening for and Using Thereof*, International Publication No. WO 02/102973; and Elisabeth Thomassen-Wolf et al., *Antibodies that Block Receptor Protein Tyrosine Kinase Activation, Methods of Screening for and Using Thereof*, International Publication No. WO 02/102854.

[0110] Other aspect of the present invention provide methods of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a SV2 wherein said selective binding reduces the ability of BoNT/A to bind to said SV2. It is envisioned that any molecule that can selectively bind to a SV2 in a manner that prevents BoNT/A binding to that same SV2 can be useful, including, without limitation, an anti-SV2 antibody, a SV2 antagonist or a SV2 agonist. In addition, a SV2, a SV2 fragment retaining BoNT/A selective binding activity or a FGFR2 and/or a FGFR3 selective binding activity, or peptidomimetic thereof can also be useful.

[0111] Aspects of the present invention provide, in part, a method of reducing BoNT/A activity in a human by administering a pharmaceutical composition comprising a molecule that selectively binds a FGFR2, a FGFR3 and/or a SV2. The administered composition can be formulated in a variety of pharmaceutically acceptable media, as described below. An effective dose of a composition disclosed in the present specification will depend upon the particular molecule selected, the route administration, and the particular characteristics of the human or other mammal, such as age, weight, general health and the like. An effective dose can be determined in an animal model prior to administration to humans. Compositions useful in aspects of the invention can be administered by a variety of routes to reducing BoNT/A activity. As a non-limiting example, oral tolerance is well-recognized in the art (see, for example, Weiner, *HOSPITAL PRACTICE*, pp. 53-58 (Sept. 15, 1995). Those skilled in the art can readily determine for a particular composition, a suitable pharmacological composition, an appropriate antigen payload; route of administration; volume of dose; and pharmaceutical regimen useful in a particular animal, for example, humans.

[0112] As disclosed herein a pharmaceutical composition is administered to a human or other mammal to reduce BoNT/A activity. As used herein, the term "reduce," when used in reference to administering to a human or other mammal an effective amount of a pharmaceutical composition, means reducing a symptom of a condition characterized by exposure to BoNT/A activity, or delaying or preventing onset of a symptom of a condition characterized by exposure to BoNT/A activity in the human or other mammal. For example, the term "reducing" can mean reducing a symptom of a condition characterized by exposure to BoNT/A activity by at least 30%, 40%, 60%, 70%, 80%, 90%, or 100%. The effectiveness of a pharmaceutical composition in treating a condition characterized by exposure to BoNT/A activity can be determined by observing one or more clinical symptoms or physiological indicators associated with the condition. An improvement in a condition characterized by exposure to BoNT/A activity also can be indicated by a reduced need for a concurrent therapy. Those of skill in the art will know the appropriate symptoms or indicators associated with specific conditions and will know how to determine if a human or

other mammal is a candidate for treatment with a pharmaceutical composition disclosed in the present specification. In particular, it is understood that those skilled in the art will be able to determine if a condition is characterized by exposure BoNT/A activity, for example, by comparison of levels of BoNT/A activity from the human or other mammal with a normal control cells.

[0113] The appropriate effective amount to be administered for a particular application of the methods can be determined by those skilled in the art, using the guidance provided herein. For example, an effective amount can be extrapolated from assays as described herein above. One skilled in the art will recognize that the condition of the patient can be monitored throughout the course of therapy and that the effective amount of a composition that is administered can be adjusted accordingly.

[0114] A pharmaceutical composition useful in aspects of the invention generally is administered in a pharmaceutically acceptable composition. As used herein, the term "pharmaceutically acceptable" refers to any molecular entity or composition that does not produce an adverse, allergic or other untoward or unwanted reaction when administered to a human or other mammal. As used herein, the term "pharmaceutically acceptable composition" refers to a therapeutically effective concentration of an active ingredient. A pharmaceutical composition may be administered to a patient alone, or in combination with other supplementary active ingredients, agents, drugs or hormones. The pharmaceutical compositions may be manufactured using any of a variety of processes, including, without limitation, conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, and lyophilizing. The pharmaceutical composition can take any of a variety of forms including, without limitation, a sterile solution, suspension, emulsion, lyophilizate, tablet, pill, pellet, capsule, powder, syrup, elixir or any other dosage form suitable for administration.

[0115] It is also envisioned that a pharmaceutical composition disclosed in the present specification can optionally include a pharmaceutically acceptable carriers that facilitate processing of an active ingredient into pharmaceutically acceptable compositions. As used herein, the term "pharmacologically acceptable carrier" refers to any carrier that has substantially no long term or permanent detrimental effect when administered and encompasses terms such as "pharmacologically acceptable vehicle, stabilizer, diluent, auxiliary or excipient." Such a carrier generally is mixed with an active compound, or permitted to dilute or enclose the active compound and can be a solid, semi-solid, or liquid agent. It is understood that the active ingredients can be soluble or can be delivered as a suspension in the desired carrier or diluent. Any of a variety of pharmaceutically acceptable carriers can be used including, without limitation, aqueous media such as, e.g., distilled, deionized water, saline; solvents; dispersion media; coatings; antibacterial and antifungal agents; isotonic and absorption delaying agents; or any other inactive ingredient. Selection of a pharmacologically acceptable carrier can depend on the mode of administration. Except insofar as any pharmacologically acceptable carrier is incompatible with the active ingredient, its use in pharmaceutically acceptable compositions is contemplated. Non-limiting examples of specific uses of such pharmaceutical carriers can be found in PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS (Howard C. Ansel et al., eds., Lippincott Williams & Wilkins Publishers, 7th ed.

1999); REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY (Alfonso R. Gennaro ed., Lippincott, Williams & Wilkins, 20th ed. 2000); GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS (Joel G. Hardman et al., eds., McGraw-Hill Professional, 10th ed. 2001); and HANDBOOK OF PHARMACEUTICAL EXCIPIENTS (Raymond C. Rowe et al., APhA Publications, 4th edition 2003). These protocols are routine procedures and any modifications are well within the scope of one skilled in the art and from the teaching herein.

[0116] It is further envisioned that a pharmaceutical composition disclosed in the present specification can optionally include, without limitation, other pharmaceutically acceptable components, including, without limitation, buffers, preservatives, tonicity adjusters, salts, antioxidants, physiological substances, pharmacological substances, bulking agents, emulsifying agents, wetting agents, sweetening or flavoring agents, and the like. Various buffers and means for adjusting pH can be used to prepare a pharmaceutical composition disclosed in the present specification, provided that the resulting preparation is pharmaceutically acceptable. Such buffers include, without limitation, acetate buffers, citrate buffers, phosphate buffers, neutral buffered saline, phosphate buffered saline and borate buffers. It is understood that acids or bases can be used to adjust the pH of a composition as needed. Pharmaceutically acceptable antioxidants include, without limitation, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene. Useful preservatives include, without limitation, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, phenylmercuric nitrate and a stabilized oxy chloro composition, for example, PURITE® (Allergan, Inc., Irvine, CA). Tonicity adjusters useful in a pharmaceutical composition include, without limitation, salts such as, *e.g.*, sodium chloride, potassium chloride, mannitol or glycerin and other pharmaceutically acceptable tonicity adjuster. The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. It is understood that these and other substances known in the art of pharmacology can be included in a pharmaceutical composition useful in the invention.

[0117] A pharmaceutical composition useful in a method of the disclosure is administered to a human or other mammal in an effective amount. Such an effective amount generally is the minimum dose necessary to achieve the desired therapeutic effect, which can be, for example, that amount roughly necessary to reduce the symptoms associated with exposure to BoNT/A activity. For example, the term "effective amount" when used with respect to treating exposure to BoNT/A activity can be a dose sufficient to the symptoms, for example, by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. Such a dose generally is in the range of 0.1-1000 mg/day and can be, for example, in the range of 0.1-500 mg/day, 0.5-500 mg/day, 0.5-100 mg/day, 0.5-50 mg/day, 0.5-20 mg/day, 0.5-10 mg/day or 0.5-5 mg/day, with the actual amount to be administered determined by a physician taking into account the relevant circumstances including the severity of the BoNT/A exposure, the age and weight of the patient, the patient's general physical condition, the cause of the BoNT/A exposure and the route of administration. Where repeated administration is used, the frequency of administration depends, in part,

on the half-life of the pharmaceutical composition. Suppositories and extended release formulations can be useful in the invention and include, for example, dermal patches, formulations for deposit on or under the skin and formulations for intramuscular injection. It is understood that slow-release formulations also can be useful in the methods of the invention. The subject receiving the pharmaceutical composition can be any mammal or other vertebrate capable of experiencing exposure to BoNT/A activity, for example, a human, primate, horse, cow, dog, cat or bird.

[0118] Various routes of administration can be useful for reducing BoNT/A activity according to a method of the invention. A pharmaceutical composition useful in the methods of the invention can be administered to a mammal by any of a variety of means depending, for example, on the type and location of BoNT/A exposure to be treated, the pharmaceutical composition, or other compound to be included in the composition, and the history, risk factors and symptoms of the subject. Routes of administration suitable for the methods of the invention include both systemic and local administration. As non-limiting examples, a pharmaceutical composition useful for reducing BoNT/A activity can be administered orally or by subcutaneous pump; by dermal patch; by intravenous, subcutaneous or intramuscular injection; by topical drops, creams, gels or ointments; as an implanted or injected extended release formulation; as a bioerodible or non-bioerodible delivery system; by subcutaneous minipump or other implanted device; by intrathecal pump or injection; or by epidural injection. An exemplary list of biodegradable polymers and methods of use are described in, e.g., HANDBOOK OF BIODEGRADABLE POLYMERS (Abraham J. Domb et al., eds., Overseas Publishers Association, 1997); CONTROLLED DRUG DELIVERY: DESIGNING TECHNOLOGIES FOR THE FUTURE (Kinam Park & Randy J. Mersny eds., American Chemical Association, 2000); Vernon G. Wong, *Method for Reducing or Preventing Transplant Rejection in the Eye and Intraocular Implants for Use Therefor*, U.S. Patent No. 6,699,493; Vernon G. Wong & Mae W. L. Hu, *Methods for Treating Inflammation-mediated Conditions of the Eye*, U.S. Patent No. 6,726,918; David A. Weber et al., *Methods and Apparatus for Delivery of Ocular Implants*, U.S. Patent Publication No. US2004/0054374; Thierry Nivaggioli et al., *Biodegradable Ocular Implant*, U.S. Patent Publication No. US2004/0137059. It is understood that the frequency and duration of dosing will be dependent, in part, on the relief desired and the half-life of the pharmaceutical composition.

[0119] In particular embodiments, a method of the invention is practiced by peripheral administration of a pharmaceutical composition. As used herein, the term "peripheral administration" or "administered peripherally" means introducing an agent into a subject outside of the central nervous system. Peripheral administration encompasses any route of administration other than direct administration to the spine or brain. As such, it is clear that intrathecal and epidural administration as well as cranial injection or implantations are not within the scope of the term "peripheral administration" or "administered peripherally."

[0120] Peripheral administration can be local or systemic. Local administration results in significantly more of a pharmaceutical composition being delivered to and about the site of local administration than to regions distal to the site of administration. Systemic administration results in delivery of a pharmaceutical

composition to essentially the entire peripheral nervous system of the subject and may also result in delivery to the central nervous system depending on the properties of the composition.

[0121] Routes of peripheral administration useful in the methods of the invention encompass, without limitation, oral administration, topical administration, intravenous or other injection, and implanted minipumps or other extended release devices or formulations. A pharmaceutical composition useful in the invention can be peripherally administered, for example, orally in any acceptable form such as in a tablet, liquid, capsule, powder, or the like; by intravenous, intraperitoneal, intramuscular, subcutaneous or parenteral injection; by transdermal diffusion or electrophoresis; topically in any acceptable form such as in drops, creams, gels or ointments; and by minipump or other implanted extended release device or formulation.

[0122] Other aspect of the present invention provide methods of screening for a molecule able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication by contacting said sample with a composition comprising an FGFR3 and detecting whether said molecule selectively binds said FGFR3, wherein selective binding of said molecule to said FGFR3 indicates that said molecule is able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication, and wherein if said molecule is BoNT/A, said method does not comprise an LD₅₀ assay. Other aspect of the present invention provide methods of screening for a molecule able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication by contacting said sample with a composition comprising a FGFR2, a FGFR3, a SV2, or any combination thereof, and detecting whether said molecule selectively binds to a FGFR2, a FGFR3 and/or a SV2, wherein selective binding of said molecule to a FGFR2, a FGFR3 and/or a SV2 indicates that said molecule is able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication, and wherein if said molecule is BoNT/A, said method does not comprise an LD₅₀ assay. As used herein, the term “selective” binding means that a binding agent is able to bind its target under physiological conditions, or *in vitro* conditions substantially approximating physiological conditions, to a statistically significantly greater degree (*i.e.*, has a smaller K_d or dissociation constant) than to other, non-target molecules on the surface of the neural cell. “K_d” is the molar concentration of the binding agent at which half the target molecules are bound by the binding agent. As used herein, the term “LD₅₀ assay” means a live animal-based *in vivo* assay of neurotoxin activity comprising detecting the dose of neurotoxin at which 50% of treated animals die, see, *e.g.*, the Mouse Protection Assay (MPA), Charles L. Hatheway & Carol Dang, *Immunogenicity of the Neurotoxins of Clostridium botulinum*, 93-107 (Neurological Disease and Therapy—THERAPY WITH BOTULINUM TOXIN, Joseph Jankovic & Mark Hallett eds., Marcel Dekker, 1994).

[0123] It is envisioned that any and all assay conditions suitable for screening for a molecule able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication can be useful, including, *e.g.*, *in vitro* and *in vivo* assays. In addition, it is also foreseen that a wide variety of processing formats can be used in conjunction with the methods disclosed in the present specification, including, without limitation, manual processing, partial automated-processing, semi-automated-processing, full

automated-processing, high throughput processing, high content processing, and the like or any combination thereof.

[0124] As disclosed above, any of the methods useful for detecting BoNT/A activity disclosed in the present specification and any of the compositions useful for practicing the methods useful for detecting BoNT/A activity disclosed in the present specification can be can be useful in screening for a molecule that competes with BoNT/A for the selectively binding to a FGFR2, a FGFR3 and/or a SV2. Thus, in aspects of this embodiment, a FGFR3 can be encoded by the nucleic acid molecule from a mammalian FGFR3, such as, *e.g.*, a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3; a bird FGFR3, such as, *e.g.*, chicken FGFR3; an amphibian FGFR3, such as, *e.g.*, a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, *e.g.*, a zebrafish FGFR3. In another aspect of this embodiment, a FGFR3 can be a mammalian FGFR3, such as, *e.g.*, a human FGFR3, a bovine FGFR3, a rat FGFR3 or a mouse FGFR3; a bird FGFR3, such as, *e.g.*, chicken FGFR3; an amphibian FGFR3, such as, *e.g.*, a newt FGFR3 or a frog FGFR3; and a fish FGFR3, such as, *e.g.*, a zebrafish FGFR3. In another aspect of this embodiment, a FGFR3 useful in screening for a molecule that competes with BoNT/A for the selectively binding to the FGFR3 can be transiently or stably contained in a cell. In another aspect of this embodiment, a FGFR2 can be encoded by the nucleic acid molecule from a mammalian FGFR2, such as, *e.g.*, a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, *e.g.*, chicken FGFR2; an amphibian FGFR2, such as, *e.g.*, a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, *e.g.*, a zebrafish FGFR2. In another aspect of this embodiment, a FGFR2 can be a mammalian FGFR2, such as, *e.g.*, a human FGFR2, a bovine FGFR2, a rat FGFR2 or a mouse FGFR2; a bird FGFR2, such as, *e.g.*, chicken FGFR2; an amphibian FGFR2, such as, *e.g.*, a newt FGFR2 or a frog FGFR2; and a fish FGFR2, such as, *e.g.*, a zebrafish FGFR2. In another aspect of this embodiment, a FGFR2 useful in screening for a molecule that competes with BoNT/A for the selectively binding to the FGFR2 can be transiently or stably contained in a cell.

[0125] In another aspect of this embodiment, a composition useful in screening for a molecule that competes with BoNT/A for the selective binding to a FGFR3 comprises a FGFR3 and optionally a SV2, such as, *e.g.*, a SV2A, a SV2B, or a SV2C. In another aspect of this embodiment, a composition useful in screening for a molecule that competes with BoNT/A for the selectively binding to a FGFR3 comprises a FGFR3 and optionally a G1b polysialoganglioside, such as, *e.g.*, GD1a, GD1b, GD3, GQ1b, or GT1b. In another aspect of this embodiment, a composition useful in screening for a molecule that competes with BoNT/A for the selective binding to a FGFR2 comprises a FGFR2 and optionally a SV2, such as, *e.g.*, a SV2A, a SV2B, or a SV2C. In another aspect of this embodiment, a composition useful in screening for a molecule that competes with BoNT/A for the selectively binding to a FGFR2 comprises a FGFR2 and optionally a G1b polysialoganglioside, such as, *e.g.*, GD1a, GD1b, GD3, GQ1b, or GT1b. In another aspect of this embodiment, a composition useful in screening for a molecule that competes with BoNT/A for the selective binding to a FGFR2 and FGFR3 comprises a FGFR2, a FGFR3, and optionally a SV2, such as, *e.g.*, a SV2A, a SV2B, or a SV2C. In another aspect of this embodiment, a composition useful in screening for a molecule that competes with BoNT/A for the selective binding to a FGFR2 and a

FGFR3 comprises a FGFR2, a FGFR3, and optionally a G1b polysialoganglioside, such as, *e.g.*, GD1a, GD1b, GD3, GQ1b, or GT1b.

[0126] In another aspect of this embodiment, a cell can include cells, such as, *e.g.*, neuronal cells including, without limitation, primary neuronal cells; immortalized or established neuronal cells; transformed neuronal cells; neuronal tumor cells; stably and transiently transfected neuronal cells expressing a FGFR2, a FGFR3, a SV2, or any combination thereof, and further include, yet are not limited to, mammalian, murine, rat, primate and human neuronal cells. Other aspects of this embodiment include cells from, such as, *e.g.*, neuronal cell lines including, without limitation, neuroblastoma cell lines, neuronal hybrid cell lines, spinal cord cell lines, central nervous system cell lines, cerebral cortex cell lines, dorsal root ganglion cell lines, hippocampal cell lines and pheochromocytoma cell lines. Non-limiting examples of neuronal cell lines include, *e.g.*, neuroblastoma cell lines BE(2)-C, BE(2)-M17, C1300, CHP-212, CHP-126, IMR 32, KELLY, LA-N-2, MC-IXC, MHH-NB-11, N18Tg2, N1E-115, N4TG3, Neuro-2a, NB41A3, NS20Y, SH-SY5Y, SiMa, SK-N-DZ, SK-N-F1, SK-N-MC and SK-N-SH; neuroblastoma/glioma hybrid cell lines N18, NG108-15 and NG115-401L; neuroblastoma/motor neuron hybrid cell lines NSC-19 and NSC-32; neuroblastoma/root ganglion neuron hybrid cell lines F11, ND-E, ND-U1, ND7/23, ND8/34 and ND27; the neuroblastoma/hippocampal neuron hybrid cell line HN-33; spinal cord cell lines TE 189.T and M4b; cerebral cortex cell lines CNh, HCN-1a and HCN-2; dorsal root ganglia cell line G4b; hippocampal cell lines HT-4, HT-22 and HN33; FGFR3 expressing cell lines H929, JIM-3, KMS-11, KMS-18, LB278, LB375, LB1017, LB2100, LP-1, OPM-2, PCL1 and UTM-2. In further aspects of this embodiment, an FGFR3 expressing cell can be, *e.g.*, H929, JIM-3, KMS-11, KMS-18, LB278, LB375, LB1017, LB2100, LP-1, OPM-2, PCL1 UTM-2, B9, TC, L6 and CFK2. Other aspects of this embodiment include cells, such as, *e.g.*, non-neuronal cells including, without limitation, primary non-neuronal cells; immortalized or established non-neuronal cells; transformed non-neuronal cells; non-neuronal tumor cells; stably and transiently transfected non-neuronal cells expressing a FGFR2, a FGFR3, a SV2, or any combination thereof, and further include, yet are not limited to, mammalian, murine, rat, primate and human non-neuronal cells. Other aspects of this embodiment include cells, such as, *e.g.*, non-neuronal cells useful in aspects of the invention further include, without limitation, anterior pituitary cells; adrenal cells, pancreatic cells, ovarian cells, kidney cells, stomach cell, blood cells, epithelial cells, fibroblasts, thyroid cells, chondrocytes, muscle cells, hepatocytes, glandular cells and cells involved in glucose transporter (GLUT4) translocation.

[0127] The molecule to be tested in the screening method may be a “small” organic compound of synthetic origin, or may be a macromolecule (either of synthetic or biological origin) including without limitation, a polypeptide, such as, *e.g.*, a growth factor, a neurotoxin, a modified neurotoxin, an antibody or an antibody derivative; a nucleic acid, such as, *e.g.*, a nucleic acid aptamer; and a polysaccharide, such as, *e.g.*, a ganglioside or a lectin. In one embodiment, the molecule is a synthetic molecule designed based on the tertiary structure and three dimensional conformation of FGF or an antibody that inhibits BoNT/A binding to a FGFR2 and/or a FGFR3. Such SAR (structure/activity relationship) analysis is routine in the art of medicinal chemistry, among other fields.

[0128] A wide variety of assays can be used to determine whether a molecule selectively binds a FGFR2, FGFR3, a SV2, or any combination thereof, including direct and indirect assays for toxin uptake. Assays that determine BoNT/A binding or uptake properties can be used to assess whether a molecule selectively binds a FGFR2, FGFR3, a SV2, or any combination thereof. Such assays include, without limitation, cross-linking assays using labeled BoNT/A, such as, e.g., BoNT/A-SBED, see, e.g., Example II of the present specification and [¹²⁵I] BoNT/A, see, e.g., Noriko Yokosawa et al., *Binding of Clostridium Botulinum Type C Neurotoxin to Different Neuroblastoma Cell Lines*, 57(1) *Infect. Immun.* 272-277 (1989); Noriko Yokosawa et al., *Binding of Botulinum Type C1, D and E Neurotoxins to Neuronal Cell Lines and Synaptosomes*, 29(2) *Toxicon* 261-264 (1991); and Tei-ichi Nishiki et al., *Identification of Protein Receptor for Clostridium Botulinum Type B Neurotoxin in Rat Brain Synaptosomes*, 269(14) *J. Biol. Chem.* 10498-10503 (1994). Other non-limiting assays include immunocytochemical assays that detect toxin binding using labeled or unlabeled antibodies, see, e.g., Atsushi Nishikawa et al., *The Receptor and Transporter for Internalization of Clostridium Botulinum Type C Progenitor Toxin into HT-29 Cells*, 319(2) *Biochem. Biophys. Res. Commun.* 327-333 (2004) and immunoprecipitation assays, see, e.g., Yukako Fujinaga et al., *Molecular Characterization of Binding Subcomponents of Clostridium Botulinum Type C Progenitor Toxin for Intestinal Epithelial Cells and Erythrocytes*, 150(Pt 5) *Microbiology* 1529-1538 (2004). Antibodies useful for these assays include, without limitation, antibodies selected against a BoNT/A, antibodies selected against a BoNT/A receptor, such as, e.g., FGFR2, FGFR3 and/or a SV2, antibodies selected against a ganglioside, such as, e.g., GD1a, GD1b, GD3, GQ1b, or GT1b and selected against a test compound, such as, e.g., a molecule that selectively binds a BoNT/A receptor wherein selective binding modulates BoNT/A activity. If the antibody is labeled, the binding of the molecule can be detected by various means, including Western blotting, direct microscopic observation of the cellular location of the antibody, measurement of cell or substrate-bound antibody following a wash step, or electrophoresis, employing techniques well-known to those of skill in the art. If the antibody is unlabeled, one may employ a labeled secondary antibody for indirect detection of the bound molecule, and detection can proceed as for a labeled antibody. It is understood that these and similar assays that determine BoNT/A uptake properties or characteristics can be useful in selecting a neuron or other cells useful in aspects of the invention.

[0129] Assays that monitor the release of a molecule after exposure to BoNT/A can also be used to assess whether a molecule selectively binds a FGFR2, FGFR3, a SV2, or any combination thereof. In these assays, inhibition of the molecule's release would occur in cells expressing a FGFR2, FGFR3, a SV2, or any combination thereof after BoNT/A treatment. As a non-limiting example the inhibition of insulin release assay disclosed in the present specification can monitor the release of a molecule after exposure to BoNT/A and thereby be useful in assessing whether a molecule selectively binds a FGFR3 (see Example I). Other non-limiting assays include methods that measure inhibition of radio-labeled catecholamine release from neurons, such as, e.g., [³H] noradrenaline or [³H] dopamine release, see e.g., A Fassio et al., *supra*, (1999); and Sara Stigliani et al., *supra*, (2003), or measures catecholamine release using a fluorometric procedure, see, e.g., Anton de Paiva et al., *supra*, (1993); Gary W. Lawrence

et al., *supra*, (1996); and Patrick Foran et al., *supra*, (1996); and methods that measure inhibition of hormone release from endocrine cells, such as, e.g., anterior pituitary cells or ovarian cells. It is understood that these and similar assays for molecule release can be useful in assessing whether a molecule selectively binds a FGFR2, FGFR3, a SV2, or any combination thereof.

[0130] As non-limiting examples, an inhibition of insulin release assay can be used to assay whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof, in FGFR2, FGFR3 and/or SV2 containing cells capable of secreting insulin; an inhibition of noradrenaline release assay can be used to assay whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof, in FGFR2, FGFR3 and/or SV2 containing cells capable of secreting noradrenaline; and an inhibition of estrogen release assay can be used to assay whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof, in FGFR2, FGFR3 and/or SV2 containing cells and capable of secreting estrogen.

[0131] Assays that detect the cleavage of a BoNT/A substrate after exposure to BoNT/A can also be used to assess whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof. In these assays, generation of a BoNT/A cleavage-product would be detected in cells expressing a FGFR2, a FGFR3, a SV2, or any combination thereof, after BoNT/A treatment. As a non-limiting example the SNAP-25 cleavage assay disclosed in the present specification can detect the cleavage of a BoNT/A substrate after exposure to BoNT/A and thereby be useful in assessing whether a molecule selectively binds a BoNT/A receptor (see Example I). Other non-limiting methods useful to detect the cleavage of a BoNT/A substrate after exposure to BoNT/A are described in, e.g., Lance E. Steward et al., *FRET Protease Assays for Botulinum Serotype A/E Toxins*, U.S. Patent 7,332,567; Lance E. Steward et al., *FRET Protease Assays for Clostridial Toxins*, U.S. Patent 7,208,285; Ester Fernandez-Salas et al., *Cell-based Fluorescence Resonance Energy Transfer (FRET) Assays for Clostridial Toxins*, U.S. Patent 7,183,006; and Ester Fernandez-Salas et al., *Immuno-Based Botulinum Toxin Serotype A Activity Assays*, U.S. Patent Application 12/403,531, each of which is hereby incorporated by reference in its entirety. It is understood that these and similar assays for BoNT/A substrate cleavage can be useful in assessing whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof.

[0132] As non-limiting examples, Western blot analysis using an antibody that recognizes BoNT/A SNAP-25-cleaved product can be used to assay whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof. Examples of anti-SNAP-25 antibodies useful for these assays include, without limitation, rabbit polyclonal anti-SNAP25₁₉₇ antiserum pAb anti-SNAP-25₁₉₇ #1 (Allergan, Inc., Irvine, CA), mouse monoclonal anti-SNAP-25 antibody SMI-81 (Sternberger Monoclonals, Lutherville, MD), mouse monoclonal anti-SNAP-25 antibody CI 71.1 (Synaptic Systems, Goettingen, Germany), mouse monoclonal anti-SNAP-25 antibody CI 71.2 (Synaptic Systems, Goettingen, Germany), mouse monoclonal anti-SNAP-25 antibody SP12 (Abcam, Cambridge, MA), rabbit polyclonal anti-SNAP-25 antiserum (Synaptic Systems, Goettingen, Germany), and rabbit polyclonal anti-SNAP-25 antiserum (Abcam, Cambridge, MA).

[0133] Assays that detect competitive binding of a molecule with BoNT/A for selective binding to a FGFR2, a FGFR3, a SV2, or any combination thereof, can also be used to assess whether a molecule selectively binds a FGFR2, a FGFR3, a SV2, or any combination thereof. In these assays, a reduction in BoNT/A activity would be detected as the amount of a molecule that competes with BoNT/A for selective binding to a BoNT/A would increase. In a non-limiting example, the competitive inhibition assay using FGF ligands disclosed in the present specification can be used to detect the competitive binding of a molecule with BoNT/A for selective binding to a FGFR3 and thereby be useful in assessing whether a molecule selectively binds a BoNT/A receptor (see Example II). Thus, in one aspect of this embodiment, competitive binding assays using a FGFR3-binding molecule with BoNT/A for selective binding to a FGFR3 can be used to assess whether a molecule selectively binds a FGFR3. In another aspect of this embodiment, competitive binding assays using a FGFR2-binding molecule with BoNT/A for selective binding to a FGFR2 can be used to assess whether a molecule selectively binds a FGFR2. In yet another aspect of this embodiment, competitive binding assays using a SV2-binding molecule with BoNT/A for selective binding to a SV2 can be used to assess whether a molecule selectively binds a SV2.

[0134] Other aspect of the present invention provide methods of rendering a cell susceptible to cleavage of SNARE proteins by BoNT/A, comprising inducing said cell to express a FGFR2, a FGFR3, a SV2, or any combination thereof. Other aspect of the present invention provide methods of transiently rendering a cell susceptible to cleavage of SNARE proteins by BoNT/A, comprising transiently inducing said cell to express a FGFR2, a FGFR3, a SV2, or any combination thereof. Other aspect of the present invention provide methods of stably rendering a cell susceptible to cleavage of SNARE proteins by BoNT/A, comprising stably inducing said cell to express a FGFR2, a FGFR3, a SV2, or any combination thereof.

[0135] Other aspect of the present invention provide methods of marketing a neurotoxin capable of selectively binding to the same FGFR2, FGFR3, SV2, or any combination thereof, as BoNT/A comprising obtaining marketing approval from a governmental or regional regulatory authority for a therapeutic neurotoxin, wherein said neurotoxin is assayed for selective binding to a cell comprising contacting said neurotoxin with a composition comprising a FGFR2, a FGFR3, a SV2, or any combination thereof, and detecting whether said neurotoxin selectively binds said FGFR2, said FGFR3, said SV2, or said combination thereof, wherein selective binding of said neurotoxin to said FGFR2, said FGFR3, said SV2, or said combination thereof indicates that said neurotoxin is able to selective binding to cells susceptible to BoNT/A intoxication and wherein if said molecule is BoNT/A, said method does not comprise an LD₅₀ assay; packaging said neurotoxin for sale in a manner consistent with the requirements of said regulatory authority, and selling said neurotoxin.

[0136] Other aspect of the present invention provide methods of marketing a neurotoxin capable of selectively binding to the same FGFR2, FGFR3, SV2, or any combination thereof, as BoNT/A comprising obtaining marketing approval from a governmental or regional regulatory authority for a therapeutic

neurotoxin, wherein said neurotoxin is assayed for selective binding to a cell comprising contacting said neurotoxin to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity; packaging said neurotoxin for sale in a manner consistent with the requirements of said regulatory authority, and selling said neurotoxin.

[0137] In another embodiment, the invention is drawn to a polypeptide comprising at least the H_C region of BoNT/A, which is produced from a bulk or formulated preparation wherein the bulk or formulated preparation is assayed for specific binding to neural cells using a method comprising contacting said polypeptide with a composition comprising a FGFR2, a FGFR3, a SV2, or any combination thereof, and, optionally, GT1b ganglioside, and detecting whether said polypeptide selectively binds FGFR2, FGFR3, SV2, or any combination thereof.

[0138] In another embodiment similar to the above aspect of the invention, the polypeptide comprises at least an FGFR2 binding domain or at least an FGFR3 binding domain, other than the H_C domain of BoNT/A. Such a binding domain may comprise, for example, an FGF, such as FGF1, FGF2, FGF4, FGF8 or FGF9, an anti-FGFR2, or an anti-FGFR3 antibody. Further, the polypeptide may optionally contain a translocation domain such as the H_N domain of BoNT/A. Additionally, the polypeptide will generally contain a clostridial neurotoxin light chain or variation thereof – the nature and/or source of the light chain can provide differences in the extent and half-life of the therapeutic effect of the polypeptide.

[0139] Thus, in this embodiment the claimed polypeptide is produced (which production may include purification, enzymatic treatment, and/or oxidation steps) from a bulk or formulation preparation. In one embodiment the preparation may be, for example, a cell lysate from fermentation of a BoNT/A-producing strain of *Clostridium botulinum*, or from a suitable mammalian, insect or bacterial host cell producing a recombinant version of BoNT/A or a recombinant modified toxin having BoNT/A binding activity. Such a bulk preparation may also be produced using cell-free transcription methodologies. In another embodiment the preparation may be purified BoNT/A formulated with associated stabilizing proteins, such as serum albumin. In each case, the preparation may comprise BoNT/A molecules which are denatured or otherwise incorrectly folded so as not to bind to the target cells. The potency and/or specific activity of the preparation, or of fractions purified from the preparation, can be detected by using the claimed assay method.

[0140] Alternatively, the polypeptide to be assayed may comprise only a portion of the entire BoNT/A molecule. For example, the bulk preparation may contain only the heavy chain of BoNT/A, as separate production of the heavy and light chains of the toxin may be a preferred way of avoiding accidental exposure to the neurotoxin by laboratory workers.

[0141] As another example of the above embodiment, the polypeptide may comprise a chimeric recombinant polypeptide which contains the H_C region of the heavy chain of BoNT/A (or some other FGFR2-binding moiety or FGFR3-binding moiety, such as FGF itself). The chimeric polypeptide comprises amino acid sequence regions additional to, or other than, those present in the wild-type BoNT/A molecule. For example, botulinum and tetanus toxins may be used as the basis for the creation of transport proteins, see, e.g., James Oliver Dolly et al., *Modification of Clostridial toxins for Use as Transport Proteins*, U.S. Patent 6,203,794. The light chain of these transport proteins are generally either replaced by a therapeutic moiety or inactivated and coupled to such a therapeutic moiety. Additionally, chimeric neurotoxins can be made comprising polypeptides containing domains of more than one neurotoxin see, e.g., James Oliver Dolly et al., *Activatable Recombinant Neurotoxins*, U.S. Patents 7,132,259 and 7,419,676, each of which is hereby incorporated by reference in its entirety. Thus, this aspect of the invention also encompasses, as a embodiment, chimeric neurotoxins containing at least the H_C domain of BoNT/A. Such molecules may be useful in modulating the time or extent of the inhibition of secretory vesicle release. Further, it may be desirable to target agents, such as therapeutic agents, to the extracellular surface of the neural cell membrane. Thus, such an agent may be joined (e.g., as a fusion protein or via post translational conjugation) to the H_C portion of BoNT/A. In such a case the cell lysate or conjugation reaction mixture may comprise a batch preparation in accordance with this aspect of the invention.

[0142] The above-referenced polypeptides are screened for binding and/or internalization essentially as mentioned above in the described screening method embodiment.

[0143] In yet another embodiment, the present invention is drawn to a method of marketing a polypeptide which contains a region capable of binding a FGFR2, a FGFR3, a SV2, or any combination thereof, comprising obtaining permission from a governmental or regional drug regulatory authority to sell said polypeptide, wherein said polypeptide is first produced from a bulk preparation which is assayed for selective binding of said polypeptide to neural cells by contacting the bulk preparation containing said polypeptide with a composition comprising FGFR2, FGFR3, SV2, or any combination thereof, and optionally GT1b ganglioside, and detecting whether said polypeptide selectively binds FGFR2, FGFR3, SV2, or any combination thereof under such conditions, packaging said polypeptide for sale in a manner consistent with the requirements of said regulatory authority, and offering said polypeptide for sale.

[0144] In this embodiment the invention is drawn to a method of marketing a polypeptide containing the H_C region of a BoNT/A toxin. The polypeptide at issue in this embodiment of the invention is produced from a bulk preparation which is assayed for purity or activity using the screening method described previously. In a step of this method, permission is obtained from a regulatory body for the marketing of such polypeptide. In this context "permission" may be tacit or express; that is, permission or approval may be obtained from the regulatory authority for the sale of a therapeutic agent or composition comprising said polypeptide, in which case "permission" is marketing approval for the sale of such agent or composition. Alternatively, "permission", as used herein, may comprise the assent, either affirmatively

given or manifested by its lack of objection, of such regulatory authority to the continued sale of a product containing a polypeptide assayed in this new manner. As before, the polypeptide may comprise BoNT/A, or a derivative thereof, or a fusion protein or conjugate containing the H_C region of the BoNT/A heavy chain.

[0145] The therapeutic product comprising the polypeptide originally contained in the bulk preparation so assayed is labeled in accordance with the requirements of the regulatory authority. The product is then offered for sale. Offering for sale may comprise advertising or sales activity, educational seminars directed at doctors, hospitals, insurers, or patients, conversations with state, regional or governmental officials concerning subsidy reimbursement (such as Medicare or Medical).

[0146] Aspects of the present invention can also be described as follows:

1. A method of detecting BoNT/A activity by contacting a sample to a cell that contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof, wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.
2. The method according to 1, wherein said cell transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.
3. The method according to 1, wherein said cell stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.
4. The method according to 1, wherein said FGFR3 is a mammalian FGFR3.
5. The method according to 4, wherein said mammalian FGFR3 is a human FGFR3, a bovine FGFR3, a mouse FGFR3, or a rat FGFR3.
6. The method according to 1, wherein said FGFR3 is a bird FGFR3.
7. The method according to 6, wherein said bird FGFR3 is a chicken FGFR3.
8. The method according to 1, wherein said FGFR3 is an amphibian FGFR3.
9. The method according to 8, wherein said amphibian FGFR3 is a frog FGFR3 or a newt FGFR3.
10. The method according to 1, wherein said FGFR3 is a fish FGFR3.

11. The method according to 10, wherein said fish FGFR3 is a zebrafish FGFR3.
12. The method according to 1, wherein said FGFR2 is a mammalian FGFR2.
13. The method according to 12, wherein said mammalian FGFR2 is a human FGFR2, a bovine FGFR2, a mouse FGFR2, or a rat FGFR2.
14. The method according to 1, wherein said FGFR2 is a bird FGFR2.
15. The method according to 14, wherein said bird FGFR2 is a chicken FGFR2.
16. The method according to 1, wherein said FGFR2 is an amphibian FGFR2.
17. The method according to 16, wherein said amphibian FGFR2 is a frog FGFR2 or a newt FGFR2.
18. The method according to 1, wherein said FGFR2 is a fish FGFR2.
19. The method according to 18, wherein said fish FGFR2 is a zebrafish FGFR2.
20. The method according to 1, wherein said SV2 is SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36.
21. The method according to 1, wherein said cell further contains a G1b polysialoganglioside.
22. The method according to 17, wherein said polysialoganglioside is selected from the group consisting of GD1a, GD1b, GD3, GQ1b, or GT1b.
23. The method according to 1, wherein said cell is a neuronal cell.
24. The method according to 23, wherein said neuronal cell is a primary neuronal cell, an immortalized neuronal cell, or a transformed neuronal cell.
25. The method according to 23, wherein said neuronal cell is selected from the group consisting of a neuroblastoma cell, a neuronal hybrid cell, a spinal cord cell, a central nervous system cell, a cerebral cortex cell, a dorsal root ganglion cell, a hippocampal cell and a pheochromocytoma cell.
26. The method according to 1, wherein said cell is a non-neuronal cell.
27. The method according to 26, wherein said non-neuronal cell is a primary non-neuronal cell, an immortalized non-neuronal cell, or a transformed non-neuronal cell.

28. The method according to 26, wherein said non-neuronal cell is selected from the group consisting of an anterior pituitary cell, an adrenal cell, a pancreatic cell, an ovarian cell, a kidney cell, a stomach cell, a blood cell, an epithelial cell, a fibroblast, a thyroid cell, a chondrocyte, a muscle cell, a hepatocyte, a glandular cell.
29. The method according to 1, wherein said sample is selected from the group consisting of a purified BoNT/A, a partially purified BoNT/A, or unpurified BoNT/A.
30. The method according to 1, wherein said sample is selected from the group consisting of a bulk BoNT/A, a formulated BoNT/A, a cosmetics BoNT/A formulation, or a clinical BoNT/A formulation.
31. The method according to 1, wherein said sample is a recombinant BoNT/A or a modified recombinant neurotoxin having BoNT/A binding activity.
32. The method according to 1, wherein said sample is selected from the group consisting of a raw food, a cooked food, a partially cooked food, or a processed food.
33. The method according to 1, wherein said sample is a sample taken from a mammal.
34. The method according to 33, wherein said mammalian sample is selected from the group consisting of a tissue, a saliva, an excretion or a feces.
35. A method of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a FGFR2 wherein said selective binding reduces the ability of BoNT/A to bind to said FGFR2.
36. A method of reducing BoNT/A activity in a human comprising administering to said human a pharmaceutical composition comprising a molecule that selectively binds a SV2 wherein said selective binding reduces the ability of BoNT/A to bind to said SV2.
37. A method of screening a for a molecule able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication by contacting said sample with a composition comprising an FGFR2, an FGFR3, a SV2, or any combination thereof, and detecting whether said molecule selectively binds said FGFR3, wherein selective binding of said molecule to said FGFR3 indicates that said molecule is able to compete with BoNT/A for selective binding to cells susceptible to BoNT/A intoxication, and wherein if said molecule is BoNT/A, said method does not comprise an LD₅₀ assay.
38. The method according to 37, wherein said contacting step is performed *in vitro* or *in vivo*.

39. The method according to 37, wherein said FGFR2 or said FGFR3 is expressed on the surface of a cell.
40. The method according to 37, wherein said cell transiently contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.
41. The method according to 37, wherein said cell stably contains an exogenous FGFR2, an exogenous FGFR3, an exogenous SV2, or any combination thereof.
42. The method according to 37, wherein said FGFR3 is a mammalian FGFR3.
43. The method according to 42, wherein said mammalian FGFR3 is a human FGFR3, a bovine FGFR3, a mouse FGFR3, or a rat FGFR3.
44. The method according to 37, wherein said FGFR3 is a bird FGFR3.
45. The method according to 44, wherein said bird FGFR3 is a chicken FGFR3.
46. The method according to 37, wherein said FGFR3 is an amphibian FGFR3.
47. The method according to 46, wherein said amphibian FGFR3 is a frog FGFR3 or a newt FGFR3.
48. The method according to 37, wherein said FGFR3 is a fish FGFR3.
49. The method according to 48, wherein said fish FGFR3 is a zebrafish FGFR3.
50. The method according to 37, wherein said FGFR2 is a mammalian FGFR2.
51. The method according to 50, wherein said mammalian FGFR2 is a human FGFR2, a bovine FGFR2, a mouse FGFR2, or a rat FGFR2.
52. The method according to 37, wherein said FGFR2 is a bird FGFR2.
53. The method according to 52, wherein said bird FGFR2 is a chicken FGFR2.
54. The method according to 37, wherein said FGFR2 is an amphibian FGFR2.
55. The method according to 53, wherein said amphibian FGFR2 is a frog FGFR2 or a newt FGFR2.

56. The method according to 37, wherein said FGFR2 is a fish FGFR2.
57. The method according to 56, wherein said fish FGFR2 is a zebrafish FGFR2.
58. The method according to 37, wherein said SV2 is SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, or SEQ ID NO: 36.
59. The method according to 37, wherein said composition further contains a G1b polysialoganglioside.
60. The method according to 59, wherein said polysialoganglioside is selected from the group consisting of GD1a, GD1b, GD3, GQ1b, or GT1b.
61. The method according to 37, wherein said cell is a neuronal cell.
62. The method according to 61, wherein said neuronal cell is a primary neuronal cell, an immortalized neuronal cell, or a transformed neuronal cell.
63. The method according to 61, wherein said neuronal cell is selected from the group consisting of a neuroblastoma cell, a neuronal hybrid cell, a spinal cord cell, a central nervous system cell, a cerebral cortex cell, a dorsal root ganglion cell, a hippocampal cell and a pheochromocytoma cell.
64. The method according to 37, wherein said cell is a non-neuronal cell.
65. The method according to 64, wherein said non-neuronal cell is a primary non-neuronal cell, an immortalized non-neuronal cell, or a transformed non-neuronal cell.
66. The method according to 64, wherein said non-neuronal cell is selected from the group consisting of an anterior pituitary cell, an adrenal cell, a pancreatic cell, an ovarian cell, a kidney cell, a stomach cell, a blood cell, an epithelial cell, a fibroblast, a thyroid cell, a chondrocyte, a muscle cell, a hepatocyte, a glandular cell.
67. The method according to any one of claims 37-39, wherein said molecule is BoNT/A.
68. The method according to 67, wherein said molecule comprises a receptor binding domain of a BoNT/A heavy chain.
69. The method according to any one of claims 37-39, wherein said molecule is a molecule that selectively binds to the receptor binding domain of FGFR2, FGFR3, SV2, or any combination thereof, and is not BoNT/A

70. The method according to 69, wherein said molecule comprises an anti-FGFR2 antibody that binds to the receptor binding domain of FGFR2, an anti-FGFR3 antibody that binds to the receptor binding domain of FGFR3, or an anti-SV2 antibody that binds to the receptor binding domain of SV2
71. The method according to 69, wherein said molecule comprises a FGF that binds to the receptor binding domain of FGFR2, FGFR3, SV2, or any combination thereof.
72. The method according to 71, wherein said FGF molecule is selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF16, FGF17, FGF18, and FGF20.
73. The method according to any one of claims 37-39, wherein said molecule is a molecule that selectively binds to the receptor binding domain of FGFR2, FGFR3, SV2, or any combination thereof, and comprises a protease domain which cleaves a SNARE protein at a site other than that cleaved by BoNT/A light chain.
74. The method according to 73, wherein said protease domain comprises the active site of the light chain of a Clostridial toxin other than BoNT/A.
75. The method according to 74, wherein said protease domain comprises the active site of the light chain of BoNT/E.
76. A method of determining BoNT/A activity from a preparation comprising BoNT/A comprising the method of 37.

EXAMPLES

[0147] The following non-limiting examples are provided for illustrative purposes only in order to facilitate a more complete understanding of the embodiments disclosed in the present specification and are in no way intended to limit any of the disclosed embodiments, which are understood to be defined solely by the claims which follow this specification.

Example I

Identification of a BoNT/A Receptor Using a Genetic Complementation Procedure

1. Identification of cells useful in screening for a BoNT/A receptor

1a. Identification of BoNT/A receptor lacking cells using an inhibition assay for insulin release

[0148] To determine whether HIT-T15 cells express a receptor for BoNT/A, an inhibition assay for insulin release was performed. In response to glucose stimulation, the hamster insulinoma cell line HIT-T15 secretes insulin in an exocytic process that depends on the activity of SNAP-25 for vesicle docking and fusion. If HIT-T15 cells lack a BoNT/A receptor, these cells would be unable to uptake BoNT/A upon exposure to this toxin and insulin secretion could occur in the presence of high glucose in the media. However, if HIT-T15 cells contain a BoNT/A receptor, insulin secretion would be inhibited after BoNT/A treatment since the toxin could intoxicate the cell and cleave SNAP-25.

[0149] To conduct an inhibition assay for insulin release, a suitable seed density of approximately 1.5×10^5 cells/mL of HIT-T15 cells was plated into individual wells of 6-well, poly-D-lysine/Laminin coated, tissue culture plates containing 3 mL of complete Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (FBS), 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 4mM Glutamine (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until the cells reach a density of about 5×10^5 cells/ml (6-16 hours). A group of HIT-T15 cells were treated with approximately 1 nM of BoNT/A by introducing the toxin using electroporation using a GENE PULSER[®] II set at 960 μ F and 0.28 kV (Bio-Rad Laboratories, Hercules, CA). An untreated control group underwent electroporation without PURE-A. The media from the wells containing treated and untreated electroporated cells was replaced with 3 mL of fresh complete DMEM supplement with either 5.6 mM glucose (low glucose) or 25 mM glucose (high glucose) and these cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately 1 hour to induce insulin secretion. The conditioned media was transferred to 15 mL tubes and the amount of insulin present in the condition media samples was determined using an Insulin ELISA assay (Peninsula Laboratories, Inc., San Carlos, CA). Exocytosis is expressed as the amount of insulin secreted per 1.5×10^5 cell/hr. Insulin release was detected in BoNT/A-untreated cells simulated by 25 mM glucose, but insulin secretion was inhibited in BoNT/A-treated cells (see FIG. 3a). These data indicate that the release of insulin in HIT-T15 cells is mediated, in part, by SNAP-25, but that these cells lack a BoNT/A receptor.

1b. Identification of BoNT/A receptor lacking cells using a SNAP-25 cleavage assay

[0150] To determine whether HIT-T15 cells express a receptor for BoNT/A, a SNAP-25 cleavage assay was performed. If HIT-T15 cells lack a BoNT/A receptor, then only the presence of the uncleaved SNAP-25 substrate would be detected after Western blot analysis. However, if HIT-T15 cells contain a BoNT/A receptor, then the toxin could intoxicate the cell and the presence of the cleaved BoNT/A SNAP-25₁₉₇ product would be detected.

[0151] To conduct a SNAP-25 cleavage assay, cells were grown in poly-D-lysine/Laminin coated 6-well plates and treated with PURE-A as described above in Example I, 1a. Cells were collected in 15 mL tubes, washed once with 1 mL of phosphate-buffered saline, pH 7.4, and then transferred to 1.5 mL microcentrifuge tubes. Cells were lysed in 0.5 mL of lysis buffer containing 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 6.8, 150 mM sodium chloride, 1.5 mM magnesium chloride, 1mM ethylene glycol *bis*(β -aminoethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA), 10% glycerol and 1% (v/v) TRITON-X[®] 100 (4-octylphenol polyethoxylate), with rotation for 1 hour at 4°C. Lysed cells were centrifuged at 5000 rpm for 10 min at 4°C to eliminate debris and the supernatants were transferred to fresh siliconized tubes. Protein concentrations were measured by Bradford's method and resuspended in 1 x SDS sample buffer at 1mg/mL or higher concentration.

[0152] To detect for the presence of a cleaved BoNT/A substrate, samples were boiled for 5 min, and 40 μ L aliquots were separated by MOPS polyacrylamide gel electrophoresis using NUPAGE[®] Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under denaturing, reducing conditions. Separated peptides were transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen, Inc, Carlsbad, CA) by Western blotting using a TRANS-BLOT[®] SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes were blocked by incubating at room temperature for 2 hours in a solution containing 25 mM Tris-Buffered Saline (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20[®], polyoxyethylene (20) sorbitan monolaureate, 2% bovine serum albumin, 5% nonfat dry milk. Blocked membranes were incubated at 4 °C for overnight in Tris-Buffered Saline TWEEN-20[®] (25 mM Tris-Buffered Saline, 0.1% TWEEN-20[®], polyoxyethylene (20) sorbitan monolaureate) containing a 1:5,000 dilution of rabbit polyclonal anti-SNAP25 antiserum pAb anti-SNAP-25₁₉₇ #1, a polyclonal antibody which is specific for the SNAP25₁₉₇-cleavage product and does not cross-react with full-length SNAP25₂₀₆, (Allergan, Inc., generated under contract with Zymed Laboratories Inc., South San Francisco, CA). Primary antibody probed blots were washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20[®]. Washed membranes were incubated at room temperature for 2 hours in Tris-Buffered Saline TWEEN-20[®] containing a 1:20,000 dilution of goat polyclonal anti-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Pierce Biotechnology, Inc., Rockford, IL) as a secondary antibody. Secondary antibody-probed blots were washed three times for 15 minutes each

time in Tris-Buffered Saline TWEEN-20[®]. Signal detection of the labeled BoNT/A SNAP25₁₉₇-cleavage product was visualized using the ECL Plus[™] Western Blot Detection System (Amersham Biosciences, Piscataway, NJ) and the membrane was imaged and cleavage product quantified with a Typhoon 9410 Variable Mode Imager and Imager Analysis software (Amersham Biosciences, Piscataway, NJ). The choice of pixel size (100 to 200 pixels) and PMT voltage settings (350 to 600, normally 400) depended on the individual blot. A BoNT/A SNAP25₁₉₇-cleavage product was detected in HIT-T15 cell treated with BoNT/A but not untreated cells, indicating that HIT-T15 cells express SNAP-25 but not the BoNT/A receptor (see FIG. 3b).

1c. Assessment of BoNT/A exposure on HIT-T15 growth

[0153] To evaluate if the presence of the toxin in the cells affect cell growth, HIT-T15 cells were electroporated as described above in Example I, 1a and monitored for 10 days. FIG. 4a demonstrates that the presence of the toxin delayed growth when compared to controls, but toxin-treated cells were able to replicate normally after a recovery period. Cell aliquots for days 3, 5, 7 and 10 were also tested for the presence of the BoNT/A SNAP-25₁₉₇ cleavage product using the SNAP-25 cleavage assay as described above in Example I, 1b. FIG. 4b shows that cleavage of SNAP-25 was detected by Western blot analysis at all time points assayed when PURE-A was introduced into the cells.

2. Identification of BoNT/A receptor using genetic complementation

[0154] To identify a BoNT/A receptor, a nucleic acid molecule encoding a BoNT/A receptor was cloned by genetic complementation. This procedure involves introducing a nucleic acid molecule encoding the BoNT/A receptor into a cell line that does not contain the receptor naturally by retroviral transduction, see, e.g., Mitchell H. Finer et al., *Methods for Production of High Titer Virus and High Efficiency Retroviral Mediated Transduction of Mammalian Cells*, U.S. Patent No. 5,858,740.

2a. Production of a retroviral stock containing pLIB expression constructs

[0155] To produce an retroviral stock containing expression constructs encoding human brain nucleic acid molecules, about 5×10^5 HEK 293-based cells (AMPHOPACK[™] 293 cells; BD Biosciences Clontech, Palo Alto, CA) were plated in 60 mm tissue culture dishes containing 5 mL of complete Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (FBS), 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 4mM Glutamine (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until the cells reach 60% to 80% confluency or a density of about 1 to 2×10^6 cells/ml (12-24 hours). On the day of transfection, the complete, supplemented DMEM media was replaced with 3 mL of OPTI-MEM[®] Reduced Serum Medium. A 500 μ L transfection solution is prepared by adding 250 μ L of OPTI-MEM[®] Reduced Serum Medium containing 15 μ L of LIPOFECTAMINE[®] 2000, a cationic liposome based reagent, (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 250 μ L of OPTI-MEM[®] Reduced Serum Medium

containing 5 µg of pLIB retroviral expression constructs containing nucleic acid molecules derived from human brain cells (BD Biosciences Clontech, Palo Alto, CA). This transfection is incubated at room temperature for approximately 20 minutes. The 500 µL transfection solution was then added to the AMPHOPACK™ 293 cells and the cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately 8-10 hours. The transfection media was replaced with 3 mL of fresh complete, supplemented DMEM and cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately 48-72 hours. The retrovirus-containing cells are harvested by detaching the cells using the culture media and scraping cells from the culture plate. Detached cells and media are transferred to a 15 mL tube and centrifuged (5,000x g at 20 °C for 15 minutes) to pellet the cellular debris. The clarified supernatant containing the retroviral particles is transferred to 2 mL cryovials in 1 mL aliquots and should contain approximately 5×10^4 to 5×10^6 pfu/mL of retroviral particles. Aliquots can be stored at -80 °C until needed.

2b. Transduction of cells with a retroviral stock containing pLIB expression constructs

[0156] To transduce cells with a retroviral stock containing expression constructs encoding human brain nucleic acid molecules, about 1.5×10^5 HIT-T15 cells were plated in 60 mm tissue culture dishes containing 5 mL of complete Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (FBS), 1x penicillin/streptomycin solution (Invitrogen, Inc, Carlsbad, CA) and 4mM Glutamine (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until the cells reach 60% to 80% confluency or a density of about 5×10^5 cells/mL (6-16 hours). Cells are inoculated with the retroviral stock containing nucleic acid molecules derived from human brain cells (see Example I, 2a), using a suitable multiplicity of infection. Approximately 4-8 µg/mL of polybrene was then added and the cells were incubated for approximately 16-24 hours in a 37 °C incubator under 5% carbon dioxide. The transduction media is replaced with 5 mL of fresh complete, supplemented DMEM and the cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately four days. The transduced cells were then used to conduct a screening assay to identify a BoNT/A receptor. For greater details on procedures described in this example, see Retroviral Gene Transfer and Expression User Manual PT3132-1 (PR43789), BD Biosciences Clontech, Palo Alto, CA, (Mar. 3, 2004).

2c. Screening of HIT-T15 cells expressing a retroviral cDNA library

[0157] To screen for cells expressing a BoNT/A receptor, transduced HIT-T15 cells as described above in Example I, 2b were screened based on their ability to bind Dynex Beads coated with BoNT/A. Approximately 7.5 mg of DYNABEADS® magnetic beads (DynaL Biotechnology, LLC, Brown Deer, WI) coated with an antibody against the light chain of BoNT/A was added to the media for 30 minutes at 4 °C and cells binding to the BoNT/A light chain of BoNT/A were isolated as clumps of cells after exposure to a magnet. These isolated cells were washed once with PBS and transferred to new 60 mm tissue culture dishes containing 5 mL of complete DMEM. These cells were re-screened with 7.5 mg of DYNABEADS® magnetic beads coated with BoNT/A for 30 minutes at 4 °C and cells binding to BoNT/A were isolated as

clumps of cells after exposure to a magnet (see FIG. 5). These re-isolated cell colonies were transferred to 96-well plates containing 0.25 mL of complete DMEM and the cells were grown in a 37 °C incubator under 5% carbon dioxide until confluent.

[0158] To test for the presence of a BoNT/A receptor, individual cells contained in the 96-well plates were assayed using the inhibition assay for insulin release assay, as described above in Example I, 1a. Cell lines containing a candidate BoNT/A receptor were selected based on the detection of the inhibition of insulin release. FIG. 6 show that transduced HIT-T15 cell lines C6 and C7 are candidate cell lines expressing a BoNT/A receptor. To confirm these results, expanded cultures of clones C6 and C7 and tested using the inhibition of insulin release assay as described above in Example I, 2a, and the SNAP-25 cleavage assay, as described above in Example I, 1b. The results indicate that a BoNT/A receptor is present in these cell lines based on the inhibition of insulin release (see FIG. 7a) and the presence of a BoNT/A SNAP25₁₉₇-cleavage product (see FIG. 7b).

2d. Cloning of BoNT/A receptor

[0159] To isolate nucleic acid molecules encoding the BoNT/A receptor, DNA will be purified from the BoNT/A receptor-containing HIT-T15 cell isolates identified above in Example I, 2c and the nucleic acid molecule encoding the BoNT/A receptor will be cloned using polymerase chain reaction (PCR) method. Genomic DNA from the C7 cell line will be isolated by an alkaline lysis procedure and will be amplified in PCR reactions using the ADVANTAGE[®] Genomic PCR kit (BD Biosciences Clontech, Palo Alto, CA) and the following two oligonucleotides 5'-AGCCCTCACTCCTTCTCTAG-3' (SEQ ID NO: 29) and 5'-ACCTACAGGTGGGGTCTTTC ATTCCC-3' (SEQ ID NO: 30). Reactions will be incubated at 95 °C for 1 minute, followed by 25 cycles at 68 °C for 30 seconds and 95 °C for 30 seconds, followed by 1 cycle at 68 °C for 6 minutes and final incubation at 4 °C. The resulting PCR product will be purified from the PCR reaction by the QIAQUICK[®] Gel Extraction Kit (QIAGEN, Inc., Valencia, CA), and will be subjected to a second PCR amplification. The oligonucleotides used in the second PCR will be nested primers designed to anneal to sequences found within the PCR product originally purified, and will have the following nucleotide sequences: 5'-CCCTGGGTCAAGCCCTTTGTACACC-3' (SEQ ID NO: 31) and 5'-TGCCAAACCTACA GGTGGGGTCTTT-3' (SEQ ID NO: 32). The resulting nested DNA product will be subcloned into a pTOPO[®]-XL vector using the TOPO[®] TA cloning method (Invitrogen, Inc, Carlsbad, CA). The ligation mixture will be transformed into chemically competent *E. coli* TOP10 cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, will be plated on 1.5% Luria-Bertani agar plates (pH 7.0) containing 100 µg/mL of Ampicillin, and will be placed in a 37 °C incubator for overnight growth. Ampicillin-resistant colonies will be analyzed using an alkaline lysis plasmid mini-preparation procedure and candidate receptor constructs will be screened by restriction endonuclease mapping to determine the presence and orientation of the correct insert fragment. Cultures containing the desired expression construct will be used to inoculate 1 L baffled flasks containing 200 mL of Luria-Bertani media containing 100 µg/mL of Ampicillin and will be placed in a 37 °C incubator, shaking at 250 rpm, for overnight growth. Purified plasmid DNA corresponding to an expression construct will be isolated using the QIAGEN Maxi-

prep method (QIAGEN, Inc., Valencia, CA) and will be sequenced to verify that the correct expression construct was made (service contract with Sequetech Corp., Mountain View, CA). This cloning strategy will identify the sequence composition of the BoNT/A receptor contained in HIT-T15 C7 isolate.

Example II

Identification of a BoNT/A Receptor Using a Cross-linking Procedure

1. Identification of cell lines with high affinity uptake for BoNT/A

[0160] Distinct sensitivities to each of the BoNT serotypes might be expected based on the individual receptor systems for each different toxin serotype and their differing expression in different cell lines. The presence of a high affinity receptor system in a cell for BoNT can be characterized by two attributes: a rapid uptake of the neurotoxin by the cell, and a low neurotoxin concentration needed for cell intoxication. To identify a cell line having a high affinity receptor system for a BoNT/A, we tested cell lines using one of two different in vitro cleavage assays, one to determine the amount of toxin required for intoxication, the other to determine the length of time necessary for the cell to uptake the neurotoxin.

1a. Assay to determine the BoNT/A concentration necessary for cell intoxication

[0161] In order to assess the amount of BoNT/A needed to intoxicate a cell, a panel of mammalian cell lines of neuronal origin (see Table 3) was screened to determine whether toxin exposure would result in the cleavage of endogenously expressed SNAP-25. A suitable seed density of cells from each line was plated into individual wells of 6-well, poly-D-lysine/Laminin coated, tissue culture plates containing 3 mL of a suitable medium (see Table 3), and grown in a 37 °C incubator under 5% carbon dioxide for approximately 24 hours. BoNT/A (Metabio, Inc., Madison, WI) was added at different concentrations (0 nM, 1 nM, 5 nM, 12.5 nM, 25 nM, 50 nM) in the culture medium containing the cells for approximately 8 or approximately 16 hours. Cells were collected in 15 mL tubes, washed once with 1 mL of phosphate-buffered saline, pH 7.4, and then transferred to 1.5 mL microcentrifuge tubes. Cells were lysed in 0.5 mL of lysis buffer containing 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 6.8, 150 mM sodium chloride, 1.5 mM magnesium chloride, 1 mM ethylene glycol *bis*(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), 10% glycerol and 1% (v/v) TRITON-X[®] 100 (4-octylphenol polyethoxylate), with rotation for 1 hour at 4 °C. Lysed cells were centrifuged at 5000 rpm for 10 min at 4 °C to eliminate debris and the supernatants were transferred to fresh siliconized tubes. Protein concentrations were measured by Bradford's method and resuspended in 1 x SDS sample buffer at 1 mg/mL or higher concentration.

[0162] The presence of a BoNT/A SNAP25₁₉₇-cleavage product was determined by Western blot analysis as described above in Example I, 1b. A BoNT/A SNAP25₁₉₇-cleavage product was detected in the cell lines SH-SY5Y, NG108-15, N1E-115, Neuro-2A and SK-N-BE(2) after at least an 8 hour

incubation with at least 5 nM BoNT/A, thereby indicating the ability of BoNT/A to intoxicate these cell lines (see FIG. 8a).

[0163] The mouse neuroblastoma cell line Neuro-2A was further analyzed with lower concentrations of BoNT/A to determine the concentration of neurotoxin necessary to cleave endogenously expressed SNAP-25. Cells were grown in poly-D-lysine/Laminin coated 6-well plates as described above in Example II, 1a. BoNT/A (Metabionics, Inc., Madison, WI) was added at different concentrations (0 nM, 0.05 nM, 0.1 nM, 0.2 nM, 0.5 nM, 1 nM, 5 nM and 20 nM) in the culture medium containing cells for either approximately 8 or approximately 16 hours. Toxin treated cells were harvested and lysed as described above in Example II, 1a. The presence of a BoNT/A SNAP25₁₉₇-cleavage product was determined by Western blot analysis as described above in Example II, 1a. A BoNT/A SNAP25₁₉₇-cleavage product was detected in the cell line Neuro-2A after at least a 8 hour incubation with at least 0.5 nM BoNT/A, thereby indicating the ability of BoNT/A to intoxicate these cell lines (see FIG. 8c).

1b. Assay to determine the time required by a cell to uptake BoNT/A

[0164] In order to assess the amount of time needed by a cell line to uptake BoNT/A, a panel of mammalian cell lines of neuronal origin was screened to determine the length of toxin exposure necessary to cleave endogenously expressed SNAP-25. Cells from each line were grown in poly-D-lysine/Laminin coated 6-well plates as described above in Example II, 1a. Approximately 1 nM BoNT/A (Metabionics, Inc., Madison, WI) was added to the culture medium for 10 min, 20 min, 30 min, 60 min 2 hours, 4 hours, 6 hours, 8 hours or 16 hours. Toxin treated cells were collected and lysed as described above in Example II, 1a. The presence of a BoNT/A SNAP25₁₉₇-cleavage product was determined by Western blot analysis as described above in Example II, 1a. A BoNT/A SNAP25₁₉₇-cleavage product was detected in the cell lines Neuro-2A, SH-SY5Y, and NG108-15 after at least an 8 hour incubation with 1 nM BoNT/A, thereby indicating the ability of these cell lines to rapidly uptake BoNT/A (see FIG. 8b).

TABLE 3
Culture Conditions for Cell Lines

Cell Line	Complete Culture Media	Passage Conditions	Seed Density (cells/mm ²)
SK-N-DZ	90% DMEM, A	Trypsin/EDTA treatment, 1:4 dilution split every 2-3 day	4.25 x 10 ³
SK-N-F1	90% DMEM, A	Trypsin/EDTA treatment, 1:4 dilution split twice a week	4.25 x 10 ³
SK-N-SH	Ham's F12, DMEM or EMEM, B	Trypsin/EDTA treatment, 1:20 dilution split every 4-7 day	4.25 x 10 ³
SH-SY5Y	EMEM and Ham's F12 1:1, C	Trypsin/EDTA treatment, 1:6 dilution split every 2-3 day	4.25 x 10 ³
SK-N-BE(2)	EMEM and Ham's F12 1:1, D	Trypsin/EDTA treatment, 1:6 dilution split every 3 day	4.25 x 10 ³
BE(2)-C	EMEM and Ham's F12 1:1, D	Trypsin/EDTA treatment, 1:4 dilution split every 2-3 day	4.25 x 10 ³
BE(2)-M17	EMEM and Ham's F12 1:1, D	Trypsin/EDTA treatment, 1:20 dilution split every 4-7 day	4.25 x 10 ³
Neuro 2a	EMEM, E	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
C1300	RPMI 1640, B	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
NB4 1A3	Ham's F10, F	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
N1E-115	DMEM, G	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
NG108-15	DMEM, B	1:4 dilution split every 1-2 days	4.25 x 10 ³
HCN-1A	DMEM, H	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
HCN-2	DMEM, H	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
TE 189.T	DMEM, H	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
ND8/34	DMEM, B	Trypsin/EDTA treatment, 1:3 dilution split every 3 day	4.25 x 10 ³
A contains 1.5g/L sodium bicarbonate, 0.1mM Non-essential amino acids (NEAA), 4mM Glutamine & 10% Fetal Calf serum (FCS) B contains 2mM Glutamine & 10% FCS C contains 1.5g/L sodium bicarbonate, 0.1mM NEAA, 4mM Glutamine, 1% sodium pyruvate, 1% penicillin/streptomycin (P/S) & 10% FCS D contains 0.1mM NEAA, 4mM Glutamine, & 10% FCS E contains 1.5g/L sodium bicarbonate, 0.1 mM NEAA, 2mM Glutamine, 1mM sodium pyruvate & 10% FCS F contains 2mM Glutamine, 15% Horse Serum & 2.5% FCS G contains 4.5g/L glucose & 10% FCS H contains 4mM glucose & 10% FCS Freezing medium comprises 95% culture medium and 5% DMSO			

1c. Ganglioside treatment to increase high affinity uptake of BoNT/A by a cell

[0165] In order to assess the effect of ganglioside treatment on the ability of BoNT/A to intoxicate a cell, a Neuro-2A cell line was pre-treated with different gangliosides to determine whether these sugar moieties could increase the uptake of BoNT/A by these cells. Neuro-2A cells were plated at a suitable density into individual wells of 6-well, poly-D-lysine/Laminin coated, tissue culture plates containing 3 mL of a suitable medium (see Table 3), and grown in a 37 °C incubator under 5% carbon dioxide. After approximately 24 hours, the medium was replaced by a serum-free media and 25 µg/mL of one of the following gangliosides was added to individual wells: GD1a, GD1b, GD3, GQ1b, or GT1b (AXXORA, LLC, San Diego, CA). After an overnight 37 °C incubation period, the ganglioside-treated cells were washed three times with 1 mL of phosphate-buffered saline, pH 7.4 and then incubated at 37 °C with 1% serum media containing different concentrations (0 pM, 12.5 pM, 25 pM, 50 pM) of BoNT/A (Metabionics, Inc., Madison, WI) for approximately 8 or approximately 16 hours. Cells were collected in 15 mL tubes, washed once with 1 mL of phosphate-buffered saline, pH 7.4, and then transferred to 1.5 mL microcentrifuge tubes. Cells were lysed in 0.5 mL of lysis buffer containing 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 6.8, 150 mM sodium chloride, 1.5 mM magnesium chloride, 1mM ethylene glycol *bis*(β-aminoethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA), 10% glycerol and 1% (v/v) Triton-X[®] 100 (4-octylphenol polyethoxylate), with rotation for 1 hour at 4 °C. Lysed cells were centrifuged at 5000 rpm for 10 min at 4 °C to eliminate debris and the supernatants were transferred to fresh siliconized tubes. Protein concentrations were measured by Bradford's method and resuspended in 1 x SDS sample buffer at 1mg/ml or higher concentration. The presence of a BoNT/A SNAP25₁₉₇-cleavage product was determined by Western blot analysis as described above in Example II, 1a. An increase in BoNT/A SNAP25₁₉₇-cleavage product was detected in the Neuro-2A cell line treated with the ganglioside GT1b, thereby indicating that GT1b-treatment can increase the uptake of BoNT/A by Neuro-2A cells (see FIG. 9a).

1d. Ganglioside treatment to increase high affinity uptake of BoNT/E by a cell

[0166] In order to assess the effect of ganglioside treatment on the ability of BoNT/E to intoxicate a cell, a Neuro-2A cell line was pre-treated with different gangliosides to determine whether these sugar moieties could increase the uptake of BoNT/E by these cells. Neuro-2A cells were grown in poly-D-lysine/Laminin coated 6-well plates and treated with gangliosides as described above in Example II, 1c. The ganglioside-treated cells were incubated with BoNT/E (Metabionics, Inc., Madison, WI) at different concentrations (0 nM, 12.5 nM, 25 nM, and 50nM) in 1% serum media for either approximately 6 or approximately 16 hours. Toxin treated cells were harvested and lysed as described above in Example II, 1c. The presence of a BoNT/E SNAP25₁₈₀-cleavage product was determined by Western blot analysis as described above in Example I, 1b, with the exception that blocked PVDF membranes were incubated in a primary antibody solution containing a 1:50,000 dilution of mouse monoclonal anti-SNAP-25 antibody (SMI-81; Sternberger Monoclonals, Lutherville, MD) rather than the rabbit polyclonal anti-SNAP25 antiserum pAb anti-SNAP-25₁₉₇ #1 and a secondary antibody solution containing a 1:20,000 dilution of

goat polyclonal anti-mouse immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Pierce Biotechnology, Inc., Rockford, IL) rather than the goat polyclonal anti-rabbit IgG-HRP antibody in order to detect a BoNT/E SNAP25₁₈₀-cleavage product. An increase in BoNT/E SNAP25₁₈₀-cleavage product was detected in the Neuro-2A cell lines treated with the gangliosides GD3, GD1b and GD1a, thereby indicating that GD3-treatment, GD1b-treatment or GD1a-treatment can increase the uptake of BoNT/E by Neuro-2A cells (see FIG. 9b).

2. Isolation of BoNT/A receptor from Neuro-2A cells

[0167] Neuro-2A cells were chosen to conduct ligand cross-linking experiments using BoNT/A since these cells had a rapid toxin uptake profile (about 10 minutes) and high affinity for BoNT/A. The trifunctional sulfo-SBED (Pierce Biotechnology, Inc., Rockford, IL) was used. The reagent sulfo-SBED contains three reactive groups (one of them designed to be UV-activated) and is designed to biotinylate a target protein.

[0168] To conjugate a cross-linking agent to a BoNT/A, approximately 100 µg of PURE-A is centrifuged at 10,000 x g at 4 °C for 10 minutes to pellet the toxin and brought up in a final volume of 900 µL of phosphate-buffered saline (pH 7.4). The solution is then transferred to the dark and 900 µL of 0.25 mM SBED, 1 % DMSO solution is added and incubated at 4 °C for two hours in a secondary container on shaking apparatus. The reaction is stopped by adding 50 µL of 1M TRIS (pH 7.4). The solution is inverted 6 times and incubated on ice for 30 minutes. The resulting PURE-A-SBED solution was used to conduct cross-linking experiments to identify a BoNT/A receptor.

[0169] To cross-link BoNT/A to a BoNT/A receptor present on Neuro-2a cells, about 1.5×10^5 Neuro-2A cells were plated in a 35 mm tissue culture dish containing 3 mL of complete EMEM, supplemented with 10% FBS, 2 mM glutamine (Invitrogen, Inc, Carlsbad, CA), 1 mM sodium pyruvate (Invitrogen, Inc, Carlsbad, CA), 1.5 g/L sodium bicarbonate and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until the cells reached a density of about 5×10^5 cells/mL. The Neuro-2A cells were harvested by detaching the cells with a trypsin treatment, transferring the cells to 15 mL tubes, and centrifuging the cells at 5,000 x g at 4 °C for 10 min. The cell pellet is washed three times with 9 mL of Tris-buffered saline, and then divided into aliquots of 4×10^8 cells. Each aliquot of cells is suspended in 12 mL cold Tris-buffered saline for a final density of 2×10^7 cells/mL, and placed on ice for 15 minutes. To one aliquot of cell suspension, 1 mL of BoNT/A-SBED is added, final concentration is approximately 100 µg BoNT/A (33nM). To a second cell aliquot, sulfo-SBED only is added and serves as a control for false positives. Both Neuro-2a cell suspensions were incubated at 4 °C for two hours in a secondary container using a shaking apparatus and then each cell solution is distributed in 13 aliquots of 1.0 mL. These aliquots were exposed to ultraviolet radiation (365 nm) at 4 °C for 15 minutes.

[0170] The cells were centrifuged at 5,000 x g at 4 °C for 15 minutes and washed once with 1 mL cold Tris-buffered saline. Washed cells were lysed in 0.5 mL of lysis buffer containing 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 6.8, 150 mM sodium chloride, 1.5 mM magnesium chloride, 1mM ethylene glycol *bis*(β -aminoethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA), 10% glycerol, 1% (v/v) Triton-X[®] 100 (4-octylphenol polyethoxylate) and suitable protease inhibitors, with rotation overnight at 4 °C. Lysed cells were centrifuged at 5,000 rpm at 4 °C for 10 min to eliminate debris, the supernatants were transferred to fresh siliconized tubes and 0.05mL of avidin-beads were added to the cleared supernatants. This mixture was incubated at 4 °C for 3 hours. The avidin beads were then washed twice by centrifuging at 1000 x g at 4 °C for 10 min to pellet beads, decanting the supernatant, adding 0.5mL lysis buffer and incubating the solution at 4 °C for 10 minutes. The avidin beads were then washed twice with 0.5mL phosphate-buffered saline (pH 7.4). Approximately 100 μ L of SDS-PAGE loading buffer was added to the washed, pelleted avidin beads and boiled for 10 minutes. A 40 μ L aliquot was then subjected to MOPS polyacrylamide gel electrophoresis using NuPAGE[®] Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under non-denaturing and denaturing, reducing conditions. FIG. 10a shows an approximately 250 kDa protein in non-reducing gels which represents the intact cross-linking reagent PURE-A-SBED toxin bound to the putative BoNT/A receptor. Same samples run under denaturing conditions and reveals an approximately 100 kDa protein was co-purified with PURE-A-SBED.

[0171] To determine the identity of the BoNT/A receptor isolated from the cross-linking experiments, western blot analysis was performed using antibodies to the cytoplasmic region of FGFR1, FGF2, FGFR3, and FGF4. Approximately 40 μ L aliquots of the precipitated receptor-PURE-A complex, obtained as described above in Example II, 2, were separated by MOPS polyacrylamide gel electrophoresis using NUPAGE[®] Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen, Inc, Carlsbad, CA) under non-reducing and denaturing, reducing conditions. Separated peptides were transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (Invitrogen, Inc, Carlsbad, CA) by Western blotting using a TRANS-BLOT[®] SD semi-dry electrophoretic transfer cell apparatus (Bio-Rad Laboratories, Hercules, CA). PVDF membranes were blocked by incubating at room temperature for 2 hours in a solution containing 25 mM Tris-Buffered Saline (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% TWEEN-20[®], polyoxyethylene (20) sorbitan monolaureate, 2% bovine serum albumin, 5% nonfat dry milk. Blocked membranes were incubated at 4 °C for overnight in Tris-Buffered Saline TWEEN-20[®] (25 mM Tris-Buffered Saline, 0.1% TWEEN-20[®], polyoxyethylene (20) sorbitan monolaureate) containing one of the following primary antibody solutions: 1) a 1:1000 dilution of rabbit polyclonal anti-FGFR1 antiserum (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA); 2) a 1:1000 dilution of goat polyclonal anti-FGFR2 antiserum (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA); 3) a 1:1000 dilution of rabbit polyclonal anti-FGFR3 (C15) antiserum (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA); or 4) a 1:1000 dilution of goat polyclonal anti-FGFR4 antiserum (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA). Primary antibody probed blots were washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20[®]. Washed membranes were incubated at room temperature for 2 hours in Tris-

Buffered Saline TWEEN-20[®] containing either a 1:20,000 dilution of goat polyclonal anti-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Pierce Biotechnology, Inc., Rockford, IL) as a secondary antibody for the FGFR1 and FGFR3 blots or a 1:20,000 dilution of rabbit polyclonal anti-goat immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Pierce Biotechnology, Inc., Rockford, IL) for the FGFR2 and FGFR4 blots. Secondary antibody-probed blots were washed three times for 15 minutes each time in Tris-Buffered Saline TWEEN-20[®]. Signal detection of the labeled BoNT/A SNAP25₁₉₇-cleavage product was visualized using the ECL PLUS[™] Western Blot Detection System (Amersham Biosciences, Piscataway, NJ) and the membrane was imaged and cleavage product quantitated with a Typhoon 9410 Variable Mode Imager and Imager Analysis software (Amersham Biosciences, Piscataway, NJ). The choice of pixel size (100 to 200 pixels) and PMT voltage settings (350 to 600, normally 400) depended on the individual blot. A band was detected in toxin-receptor sample probed with anti-FGFR3 antiserum of approximately 97 kDa that is consistent with the size of FGFR3, indicating that FGFR3 is a BoNT/A receptor (see FIG. 10b).

3. Identification of BoNT/A receptor from various cells

[0172] Several cells lines responsive to BoNT/A uptake were probed with antibodies raised against FGFR1, FGFR2, FGFR3 and FGFR4 in order to determine which FGFRs these cell lines express. In addition, cells from the BoNT/A unresponsive HIT-T15 wild-type cell line and the BoNT/A responsive HIT-T15 isolate C7 cell line, as described above in Example I, 2c and 2d, were examined.

[0173] To determine the presence of FGFRs in cell lines responsive to BoNT/A exposure, cells were grown, harvested and lysed as described above in Example II, 1a,1b or 2c and 40 μ L aliquots were subjected to Western blot analysis as described above in Example II, 2. These results indicate that the BoNT/A responsive cell lines Neuro-2A, SH-SY5Y and HIT-T15-C7 all express FGFR3, while the BoNT/A unresponsive wild-type HIT-T15 does not (see FIG. 11). The data also from the revealed that FGFR4 were not detected in any of the cell lines tested, while FGFR1 was present in all cell lines tested, including wild-type HIT-T15 cells that are unresponsive to BoNT/A exposure (see FIG. 11).

4. Competitive competition assays

[0174] To corroborate that BoNT/A toxin enters Neuro-2A cells through FGFR3 a competition experiment was performed with BoNT/A and the ability of the toxin to internalize into the cell was accessed by using the SNAP-25 cleavage assay, as described above in Example I, 1b. If BoNT/A and an FGFR3 ligand bind to the same receptor, then increasing amounts of FGF ligand should result in decreased responsiveness of a cell to BoNT/A exposure. However, if BoNT/A and an FGFR3 ligand bind to the different receptors, then increasing amounts of FGF ligand should have no effect of the responsiveness of a cell to BoNT/A exposure. Table 1, which Applicants do not claim is a complete

tabulation of FGF receptors and ligands, shows certain members of the family of FGFRs and their known ligands and tissue distribution.

[0175] To determine whether ligands for FGFR3 can competitively compete with BoNT/A for binding to FGFR3, about 5×10^5 Neuro-2A cells were plated in individual wells of a 6-well, poly-D-lysine/Laminin coated, tissue culture plates containing 3 mL of EMEM, supplemented with 2 mM glutamine (Invitrogen, Inc, Carlsbad, CA), 1 mM sodium pyruvate (Invitrogen, Inc, Carlsbad, CA), 1.5 g/L sodium bicarbonate and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until the cells reached confluency. Approximately 1-5 nM BoNT/A (Metabio, Inc., Madison, WI) was added in conjunction with FGF1, FGF2, FGF9, FGF10, or both FGF1 and FGF2 at different concentrations (0 nM, 0.1 nM, 1 nM, 5 nM, 50 nM, 200 nM) in the culture medium containing the cells and incubated at 37 °C for approximately 10 minutes. rHC/A and Fc-IgG were used as positive and negative controls respectively. Cells were collected in 15 mL tubes, washed once with 1 mL of phosphate-buffered saline, pH 7.4, and then transferred to 1.5 mL microcentrifuge tubes. Cells were lysed in 0.5 mL of lysis buffer containing 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 6.8, 150 mM sodium chloride, 1.5 mM magnesium chloride, 1mM ethylene glycol *bis*(β -aminoethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA), 10% glycerol and 1% (v/v) TRITON-X[®] 100 (4-octylphenol polyethoxylate), with rotation for 1 hour at 4 °C. Lysed cells were centrifuged at 5000 rpm for 10 min at 4 °C to eliminate debris and the supernatants were transferred to fresh siliconized tubes. Protein concentrations were measured by Bradford's method and resuspended in 1 x SDS sample buffer at 1mg/ml or higher concentration.

[0176] The presence of a BoNT/A SNAP25₁₉₇-cleavage product was determined by Western blot analysis as described above in Example II, 1a, with the exception that blocked PVDF membranes will be incubated in a primary antibody solution containing a 1:50,000 dilution of mouse monoclonal anti-SNAP-25 antibody (SMI-81; Sternberger Monoclonals, Lutherville, MD) rather than the rabbit polyclonal anti-SNAP25 antiserum pAb anti-SNAP-25₁₉₇ and a secondary antibody solution containing a 1:20,000 dilution of goat polyclonal anti-mouse immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Pierce Biotechnology, Inc., Rockford, IL) rather than the goat polyclonal anti-rabbit IgG-HRP antibody in order to detect both the uncleaved SNAP-25 substrate and BoNT/A SNAP25₁₉₇-cleavage product. An increasing amount of FGF ligands indicated that FGF1 and FGF2 compete for the same receptor as BoNT/A and further confirming that FGFR3 is a BoNT/A receptor (see FIG. 12a). Another receptor competition experiment indicated that FGF2 and FGF9, but not FGF10, effectively competed with BoNT/A for binding to the BoNT/A receptor (see FIG. 12b).

Example III

[0177] A fusion protein comprising the C terminal portion of the heavy chain of BoNT/A and the light chain of BoNT/E, or comprising the LH_N/E (LC/E plus H_N/E) and the binding domain of BoNT/A (H_C/A), are tested for their ability to selectively bind and intoxicate BoNT/A susceptible cells. A preparation

comprising dilutions of the fusion proteins are incubated with HIT-T15 insulinoma cells expressing exogenous FGFR3 in the presence of GT1b ganglioside. The ability of the fusion peptides to bind and enter the insulinoma cells is detected by detecting secretion of insulin in response to the presence of glucose, as described above in Example I, 1a. By contrast, insulin secretion is unaffected in cells not expressing FGFR3.

[0178] The results of this assay show that the amount of insulin secreted into the culture medium is decreased in a dose-dependent manner when the fusion proteins are added to the culture medium. Western blots of cell lysates will show the conversion of full length SNAP-25 to the cleaved form typical of the proteolytic activity of the BoNT/E light chain protease SNAP-25₁₈₀. This assay therefore is useful in showing that the fusion peptides are able to bind and enter BoNT/A susceptible cells.

[0179] The same fusion proteins are capable of intoxicating cells of the neuromuscular junction.

Example IV

[0180] A fusion protein comprising the receptor binding portion of an FGF species capable of binding FGFR3 (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF16, FGF17, FGF18, and FGF20) and the translocation domain and light chain of BoNT/E is tested for its ability to selectively bind and intoxicate BoNT/A susceptible cells. The assay is conducted as described in Example 1 above, with similar results; the detected cleaved SNAP-25 fragments are characteristic of BoNT/E intoxication.

Example V

[0181] BoNT/A, produced from fermentation of *Clostridium botulinum* is produced using standard fermentation techniques. Either or both the bulk preparation and purified, formulated versions of expressed toxin are tested for purity and activity as follows. A preparation comprising dilutions of the BoNT/A preparation is incubated with HIT-T15 insulinoma cells expressing exogenous FGFR3 in the presence of GT1b ganglioside. The ability of the toxin to bind and enter the insulinoma cells is detected by detecting secretion of insulin in response to the presence of glucose, as described above in Example I, 1a. The specific activity of the preparation can be calculated from the determined protein concentration and the activity of the preparation at various doses.

[0182] These data are submitted to the U.S. Food and Drug Administration by a pharmaceutical company as part of data demonstrating how BoNT/A is manufactured and tested. This information is considered by the FDA, who decides to permit the manufacture and sale of this lot of BoNT/A, and subsequent lots made and tested in a similar manner, as a therapeutic pharmaceutical product based in part on this bulk and/or formulation assay data.

[0183] The pharmaceutical comprising the BoNT/A is then offered for sale as a prescription medication.

Example VI

[0184] Same as Example V, however the polypeptide produced is the fusion neurotoxin of Example III, produced in *E. coli*. Both bulk and/or formulation lots of the fusion neurotoxin are tested as indicated above, the data submitted to the FDA, and a decision to grant marketing approval, or continued sales of such fusion polypeptide as a therapeutic agent, is made by the FDA based at least in part on such data. The pharmaceutical company then offers the fusion neurotoxin for sale as a prescription therapeutic agent.

Example VII

[0185] An *in vitro* assay is established using an FGFR3 bound to a solid support in the presence of ganglioside GT1b. The bound FGFR3 is first saturated with BoNT/A heavy chain (H chain) in phosphate buffered saline (PBS), and washed free of unbound FGF. A test compound from a combinatorial library of compounds is contacted with the receptor under substantially physiological conditions (e.g., PBS), and the elute collected. The H chain concentration in the elute is compared to the H chain concentration of a control elute in which H chain was not first bound to FGFR3.

[0186] Test compounds which are able to strongly bind FGFR3 and compete with H chain for FGFR3 binding (for example, by the method described in this section) are candidates compounds for the development of an antidote to acute botulism poisoning.

Example VIII

Generation of cells stably containing a FGFR3

1. Construction of pQBI25/FGFR3

[0187] To construct pQBI-25/FGFR3, a nucleic acid fragment encoding the amino acid region comprising FGFR3 of SEQ ID NO: 4 is amplified from a human brain cDNA library using a polymerase chain reaction method and subcloned into a pCR2.1 vector using the TOPO[®] TA cloning method (Invitrogen, Inc, Carlsbad, CA). The forward and reverse oligonucleotide primers used for this reaction are designed to include unique restriction enzyme sites useful for subsequent subcloning steps. The resulting pCR2.1/FGFR3 construct is digested with restriction enzymes that 1) excise the insert containing the entire open reading frame encoding the FGFR3; and 2) enable this insert to be operably-linked to a pQBI-25 vector (Qbiogene, Inc., Carlsbad, CA). This insert is subcloned using a T4 DNA ligase procedure into a pQBI-25 vector that is digested with appropriate restriction endonucleases to yield pQBI-25/FGFR3. The ligation mixture is transformed into chemically competent *E. coli* BL21 (DE3) cells (Invitrogen, Inc, Carlsbad, CA) using a heat shock method, plated on 1.5% Luria-Bertani agar plates (pH

7.0) containing 100 µg/mL of Ampicillin, and placed in a 37 °C incubator for overnight growth. Bacteria containing expression constructs are identified as Ampicillin resistant colonies. Candidate constructs are isolated using an alkaline lysis plasmid mini-preparation procedure and analyzed by restriction endonuclease digest mapping to determine the presence and orientation of the inset. This cloning strategy yields a mammalian expression construct encoding the FGFR3 of SEQ ID NO: 4 operably-linked to the expression elements of the pQBI-25 vector.

2. Stably transformed cells using a recombinant crossing-over procedure

[0188] To generate a stably-integrated cell line expressing a FGFR3 using a crossing over procedure, a suitable density (1×10^5 to 1×10^6 cells) of appropriate cells, such as, *e.g.*, HIT-T15 or Neuro2A, are plated in a 35 mm tissue culture dish containing 3 mL of complete, supplemented culture media and grown in a 37 °C incubator under 5% carbon dioxide until the cells reached a density appropriate for transfection. A 500 µL transfection solution is prepared by adding 250 µL of OPTI-MEM[®] Reduced Serum Medium containing 15 µL of LIPOFECTAMINE[®] 2000, a cationic liposome based reagent, (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 250 µL of OPTI-MEM[®] Reduced Serum Medium containing 5 µg of expression construct encoding a FGFR3, such as, *e.g.*, pQBI-25/FGFR3 (see Examples VIII, 1). This transfection was incubated at room temperature for approximately 20 minutes. The complete, supplemented media is replaced with 2 mL of OPTI-MEM[®] Reduced Serum Medium and the 500 µL transfection mixture is added to the cells and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 16 hours. Transfection media is replaced with 3 mL of fresh complete, supplemented culture media and the cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 48 hours. Media is replaced with 3 mL of fresh complete, supplemented culture media, containing approximately 5 µg/mL of G418. Cells are incubated in a 37 °C incubator under 5% carbon dioxide for approximately 4 weeks, with old media being replaced with fresh G418 selective, complete, supplemented media every 4 to 5 days. Once G418-resistant colonies are established, resistant clones are replated to new 35 mm culture plates containing fresh complete culture media, supplemented with approximately 5 µg/mL of G418 until these cells reached a density of 6 to 20×10^5 cells/mL.

[0189] To test for expression of a FGFR3 from isolated cell lines that stably-integrated an expression construct encoding a FGFR3, such as, *e.g.*, pQBI-25/FGFR3 (see Examples VIII, 1), approximately 1.5×10^5 cells from each cell line are plated in a 35 mm tissue culture dish containing 3 mL of G418-selective, complete, supplemented DMEM and are grown in a 37 °C incubator under 5% carbon dioxide until cells reached a density of about 5×10^5 cells/ml (6-16 hours). Media is replaced with 3 mL of fresh G418-selective, complete, supplemented culture media and cells are incubated in a 37 °C incubator under 5% carbon dioxide. After 48 hours, the cells are harvested by rinsing the cells once with 3.0 mL of 100 mM phosphate-buffered saline, pH 7.4 and are lysed with a buffer containing 62.6 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl), pH 6.8 and 2% sodium lauryl sulfate (SDS). Lysed cells are centrifuged at 5000 rpm for 10 min at 4°C to eliminate debris and the supernatants are

transferred to fresh siliconized tubes. Protein concentrations are measured by Bradford's method and are resuspended in 1 x SDS sample buffer at 1mg/ml or higher concentration.

[0190] To detect for the presence of a FGFR3, samples are separated by MOPS polyacrylamide gel electrophoresis and analyzed by Western blotting procedures as described above in Example II, 2 using a 1:1000 dilution of rabbit polyclonal anti-FGFR3 (C15) antiserum (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA), in order to identify cell lines that have stably integrated and express the FGFR3.

Example IX **FGFR3 Phosphorylation Studies**

1. Phosphorylation of FGFR-3 exposed to FGF or BoNT/A

[0191] When bound by specific ligands, FGFR's are auto-phosphorylated on specific tyrosine residues. This begins the process of internalization of both the receptor and the ligand into the endosomal pathway. If BoNT/A binds to FGFR3, then exposure to BoNT/A should cause the auto-phosphorylation of FGFR3 in exposed cells.

[0192] To determine whether BoNT/A binding resulted in FGFR3 phosphorylation, approximately 1.5×10^5 Neuro-2A cells were plated into the wells of 6-well, poly-D-lysine/Laminin coated, tissue culture plates containing 3 mL of serum-free EMEM, supplemented with 1 mM sodium pyruvate (Invitrogen, Inc, Carlsbad, CA), 1.5 g/L sodium bicarbonate and 1x MEM non-essential amino acids solution (Invitrogen, Inc, Carlsbad, CA), and grown in a 37 °C incubator under 5% carbon dioxide until the cells reached a density of about 5×10^5 cells/ml. The serum-free media was replaced with fresh supplemented EMEM containing 1 % FBS (Invitrogen, Inc, Carlsbad, CA) and either 5 nM FGF-2 (Biosource International, Camarillo, CA) or 5 nM of PURE/A (Metabio, Inc., Madison, WI). The cells were then incubated in a 37 °C incubator under 5% carbon dioxide for approximately 5 min, 10 min, 20 min and 30 min, with unexposed cells used as time 0. Cells were collected in 15 mL tubes, washed once with 1 mL of phosphate-buffered saline, pH 7.4, and then transferred to 1.5 mL microcentrifuge tubes. Cells were lysed in 0.5 mL of lysis buffer containing 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES), pH 6.8, 150 mM sodium chloride, 1.5 mM magnesium chloride, 1mM ethylene glycol *bis*(β -aminoethyl ether) *N, N, N', N'*-tetraacetic acid (EGTA), 10% glycerol and 1% (v/v) TRITON-X[®] 100 (4-octylphenol polyethoxylate), with rotation for 1 hour at 4°C. Lysed cells were centrifuged at 5000 rpm for 10 min at 4 °C to eliminate debris and the supernatants were transferred to fresh siliconized tubes. Protein concentrations were measured by Bradford's method and resuspended in 1 x SDS sample buffer at 1mg/mL or higher concentration.

[0193] Supernatant containing 100 μ g of protein was immunoprecipitated using 5 μ g of anti-phosphotyrosine antibody attached to a sepharose bead (Zymed Laboratories, Inc., South San Francisco, CA). The immunoprecipitated product were subjected to Western blot analysis as described

above in Example II, 4, with the blots being probed for FGFR3 (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA). These experiments show that FGFR3 is phosphorylated upon either FGF2 or BoNT/A exposure, indicating that BoNT/A binds to FGFR3 (see FIG. 13a).

2. DMBI Inhibition of FGFR-3 phosphorylation exposed to FGF

[0194] To determine whether DMBI inhibits BoNT/A-induced FGFR3 phosphorylation, Neuro-2A cells were plated and grown as described above in Example IX, 1. Neuro-2A cells were plated at a density of 5×10^5 cells/well (6 well plate) and incubated overnight in serum-free media. The media was replaced with fresh serum-free supplemented EMEM containing 0, 1 μ M, 5 μ M, 20 μ M, or 100 μ M of DMBI (EMD Calbiochem, San Diego, CA) for 1 hour. DMBI inhibits the autophosphorylation and dimerization of FGFR and PDGF type receptors. The cells were then washed and fresh supplemented EMEM containing 1 % FBS (Invitrogen, Inc, Carlsbad, CA) and 5 nM FGF-2 (Biosource International, Camarillo, CA). The cells were then incubated in a 37 °C incubator under 5% carbon dioxide for approximately 5 min, 10 min and harvested and immunoprecipitated as described above in Example IX, 1. The immunoprecipitated products were subjected to Western blot analysis as described above in Example II, 4, with the exception that the blots were probed with a primary antibody solution containing a 1:1000 dilution of a rabbit polyclonal anti-phosphotyrosine antiserum (Upstate USA, Inc., Charlottesville, VA) and a secondary antibody solution containing a 1:20,000 dilution of goat polyclonal anti-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Pierce Biotechnology, Inc., Rockford, IL). These results indicate that DMBI effectively inhibits the phosphorylation of FGFR3 upon FGF2 exposure (see FIG. 13b).

3. DMBI Inhibition of BoNT/A activity

[0195] To determine whether DMBI can inhibit BoNT/A activity, Neuro-2A cells were plated and grown as described above in Example IX, 1. The media was replaced with fresh serum-free supplemented EMEM containing 0, 1 μ M, 5 μ M, 20 μ M, or 100 μ M of DMBI (EMD Calbiochem, San Diego, CA) for 1 hour. DMBI inhibits the autophosphorylation and dimerization of FGFR and PDGF type receptors. The cells were then washed and fresh supplemented EMEM containing 1 % FBS (Invitrogen, Inc, Carlsbad, CA) and 5 nM of PURE/A (Metabio, Inc., Madison, WI). The cells were then incubated in a 37 °C incubator under 5% carbon dioxide for approximately 5-10 min and harvested as described above in Example IX, 1. Aliquots were tested for the presence of the BoNT/A SNAP-25₁₉₇ cleavage product using the SNAP-25 cleavage assay as described above in Example I, 1b. These results indicate a reduction in the amount of SNAP-25 cleavage product present, thereby indicating that DMBI effectively inhibits BoNT/A activity and confirming that this toxin is internalized by FGFR3 (see FIG. 13c).

Example X

BoNT/A Binding to FGFR2 and FGFR3

[0196] To determine which BoNT/A receptors BoNT/A binds to, a SNAP-25 cleavage assay was performed using SiMa, PC-12 and Neuro-2a cells that were transiently transfected with FGFR1, FGFR2, FGFR3, and/or SV2. The following mammalian expression constructs for each of the receptors were made using routine molecular biology sub-cloning techniques: pReceiver-M02/FGFR1, pReceiver-M02/FGFR2, pReceiver-M02/FGFR3, pReceiver-M02/SV2C, and pDNA6.2/V5-DEST/SV2C.

[0197] To transiently transfect the cells, about 5×10^6 cells were plated in 100 mm poly-D-lysine tissue culture plates containing 5 mL of the appropriate buffer and grown in a 37 °C incubator under 5% carbon dioxide until the cells reached 60% to 80% confluency or a density of about 1 to 2×10^6 cells/ml (12-24 hours). For SiMa cells the growth media comprises RPMI 1640 (Invitrogen, Inc., Carlsbad, CA), 10% FBS (Invitrogen, Inc., Carlsbad, CA), 10 mM HEPES (Invitrogen, Inc., Carlsbad, CA), 1 mM Sodium Pyruvate (Invitrogen, Inc., Carlsbad, CA), 0.1 mM Non-Essential Amino Acids (Invitrogen, Inc., Carlsbad, CA), 100 U/μL Penicillin (Invitrogen, Inc., Carlsbad, CA), and 100 μg/mL Streptomycin (Invitrogen, Inc., Carlsbad, CA). For PC-12, the growth media comprised RPMI 1640, 10% Equine Serum (Invitrogen, Inc., Carlsbad, CA), 5% FBS, 10 mM HEPES, 1 mM Sodium Pyruvate, 2 mM GLUTAMAX™ (glutamine supplement) with Earle's salts (Invitrogen, Inc., Carlsbad, CA), 100 U/μL Penicillin, and 100 μg/mL Streptomycin. For Neuro-2a cells, the growth media comprised Minimum Essential Media (Invitrogen, Inc., Carlsbad, CA), 10% FBS, 10 mM HEPES, 1 mM Sodium Pyruvate, 2 mM GLUTAMAX™, 0.1 mM Non-Essential Amino Acids, 100 U/μL Penicillin, and 100 μg/mL Streptomycin. On the day of transfection, the complete media was replaced with 5 mL of OPTI-MEM® Reduced Serum Medium (Invitrogen, Inc., Carlsbad, CA). A 3.0 mL transfection solution is prepared by adding 1.5 mL of OPTI-MEM® Reduced Serum Medium containing 60 μL of LIPOFECTAMINE® 2000, a cationic liposome based reagent, (Invitrogen, Carlsbad, CA) incubated at room temperature for 5 minutes to 1.5 mL of OPTI-MEM® Reduced Serum Medium containing 24 μg of the appropriate expression constructs containing nucleic acid molecules encoding the receptor or receptors to be tested. This transfection is incubated at room temperature for approximately 30 minutes. The 3.0 mL transfection solution was then added to the cells and the cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately 8 hours. The transfection media was replaced with 5 mL of fresh complete media and cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately 24 hours prior to selection in media containing 1 mM G418 (Invitrogen, Inc., Carlsbad, CA) or 2.0 μg/mL Blastidin (Invitrogen, Inc., Carlsbad, CA). After about one week of antibiotic selection, the cells were then grown in media containing 0.5 mM G418 or 1.0 μg/mL Blastidin and maintained indefinitely by serial passaging.

[0198] After the transfected cells grew to an appropriate density, the cells were trypsin-treated to produce a cell suspension and the cell concentration was determined. About 5×10^4 cells were plated into the wells of poly-D-lysine coated 96-well cell culture plates containing 100 μL of an appropriate differentiation medium containing 25 μg/mL GT1b (Alexis Biochemicals, San Diego, CA). For SiMa cells, the differentiation media comprised Minimum Essential Media, 10 mM HEPES, 2 mM GLUTAMAX™ (glutamine supplement) with Earle's salts, 0.1 mM Non-Essential Amino Acids, 1 x N2 supplement (Invitrogen, Inc., Carlsbad, CA), and 1 x B27 supplement (Invitrogen, Inc., Carlsbad, CA). For PC-12

cells the differentiation media comprised RPMI 1640, 10 mM HEPES, 1 mM Sodium Pyruvate, 2 mM GLUTAMAX™, 1 x N2 supplement, and NGF (Promega Corp., Madison, WI). For Neuro-2a cells the differentiation media comprised Minimum Essential Media, 10 mM HEPES, 2 mM GLUTAMAX™ with Earle's salts, 0.1 mM Non-Essential Amino Acids, 1 x N2 supplement, and 1 x B27 supplement. The cells were incubated in a 37 °C incubator under 5% carbon dioxide until the cells differentiated, as assessed by standard and routine morphological criteria, such as growth arrest and neurite extension (approximately 3 days). After differentiation, the media was aspirated from the plate and replaced with fresh differentiation media containing either 0 (untreated sample), 0.9 nM, 1.4 nM, 1.9 nM, 3.7 nM, 9.0 nM, 33 nM, and 90 nM of a BoNT/A complex. The cell lines were treated for 6 hours, washed, and incubated for an additional 15 hours in differentiation media. To harvest the cells, the media was aspirated, the cells were lysed by adding 40 µL of 2 x SDS Loading Buffer, the lysate was transferred to a clean 96-well plate tube and the samples was heated to 95 °C for 5 minutes.

[0199] The presence of a BoNT/A SNAP25₁₉₇-cleavage product was determined by Western blot analysis as described above in Example II, 1a, except that harvested samples are separated by SDS-PAGE using 12 % 26-well Criterion gels (Bio-Rad Laboratories, Hercules, CA, the blocked PVDF membranes were incubated in a primary antibody solution containing a 1:25,000 dilution of rabbit polyclonal anti-SNAP-25 antibody (9684; Sigma, Corp., St. Louis, MO), and a secondary antibody solution containing a 1:20,000 dilution of goat polyclonal anti-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (HRP; Invitrogen, Inc., Carlsbad, CA). The results indicate that cells transfected with FGFR2 or FGFR3 were more sensitive to BoNT/A intoxication than cells transfected with SV2C, FGFR1, or vector alone (Table 4). Neuro-2a cell and PC12 cells transfected with FGFR3c had a higher maximum signal (Higher efficiency) and a lower EC₅₀ (Higher potency) for BoNT/A than the cells transfected with SV2C or empty vector and the cells transfected with both SV2C and FGFR3c had a similar response to BoNT/A as cells transfected with FGFR3c alone (Table 4). This was also true when FGFR3c was transfected into SiMa and SH-SY-5Y human neuroblastoma cells.

TABLE 4. EC₅₀ of Cell Lines Transfected with Receptors			
Cell Line	Construct	EC₅₀ / Sensitivity	Signal_{MAX}/Efficacy
SiMa	Empty Vector	3.96 pM	17938745
SiMa	FGFR3c	1.71 pM	18714070
SiMa	SV2C	2.54 pM	17980114
SiMa	FGFR3 & SV2C	1.3 pM	20409337
PC-12	Empty Vector	5.5 nM	783235
PC-12	FGFR3c	3.1 nM	1636910
PC-12	SV2C	4.3 nM	109326
PC-12	FGFR3 & SV2C	2.5 nM	1764551
Neuro-2a	Empty Vector	0.958 nM	12127623
Neuro-2a	FGFR3c	0.53 nM	15620766

Neuro-2a	SV2C	0.72 nM	11328029
Neuro-2a	FGFR3 & SV2C	0.51 nM	14603272

[0200] Expression plasmids containing FGFR1c, FGFR2c, or FGFR3c were transfected into three different neuronal cell lines, SiMa, PC-12, and Neuro-2a. Over-expression of FGFR1c, FGFR2c, or FGFR3c was confirmed by Western blotting. The sensitivity of the transfected cells to BoNT/A was determined using a Western blot assay that measures the amount of cleaved SNAP-25₁₉₇ after treatment with BoNT/A as described above. The EC₅₀ for each of the cells lines was estimated using SigmaPlot software. For the estimate to be accurate the response has to reach an upper asymptote, this was only the case for the SiMa cells and for the PC-12 cells expressing one of the receptors.

[0201] Cells transfected with FGFR2c or FGFR3c were more sensitive to BoNT/A than cells transfected with empty vector alone. FGFR1c only increased uptake in PC-12 cells (Figure 15, Table 5). The EC₅₀ values for cells over-expressing FGFR3c was lower than the EC₅₀ values for cells over-expressing FGFR2c. In SiMa cells the EC₅₀ for cells over-expressing FGFR2c was estimated to 45.9 pM, while the EC₅₀ for cells over-expressing FGFR3c was estimated to 37.7 pM. For comparison, the EC₅₀ for SiMa cells expressing only GFP was estimated to 81.2 pM. FGFR1c only significantly increased uptake in PC-12 cells and here the EC₅₀ value for cells over-expressing FGFR1c was higher than for cells over-expressing either FGFR2c or FGFR3c (565.0 pM for FGFR1c versus 342.9 pM for FGFR2c and 263.4 pM for FGFR3c). The results indicate that cells transfected with FGFR2 or FGFR3 were more sensitive to BoNT/A intoxication than cells transfected with SV2C, FGFR1, or vector alone.

TABLE 5. Sensitivity and Efficacy of Cell Lines Transfected with FGFR Receptors

Cell Line	Construct	EC ₅₀ (nM) / Sensitivity	Signal _{MAX} / Efficacy
SiMa	GFP Vector	0.0812 +/- 0.0096	22733787
SiMa	FGFR1	0.0809 +/- 0.0089	30032360
SiMa	FGFR2	0.0459 +/- 0.0030	26136578
SiMa	FGFR3	0.0377 +/- 0.0062	24326271
PC-12	GFP Vector	0.5650 +/- 0.1964	25313955
PC-12	FGFR1	0.3429 +/- 0.0594	25376114
PC-12	FGFR2	0.2634 +/- 0.0255	24102459
PC-12	FGFR3	61.80 +/- 9.71	4605974
Neuro-2a	GFP Vector	31.59 +/- 8.8	23279765
Neuro-2a	FGFR1	11.55 +/- 5.24	28347413
Neuro-2a	FGFR2	0.0812 +/- 0.0096	22733787
Neuro-2a	FGFR3	0.0809 +/- 0.0089	30032360

[0202] To determine the binding of a receptor to BoNT/A, binding assays were performed on a BIAcore 3000 instrument using carboxymethyl dextran (CM5) sensor chips (BIAcore, Inc., Piscataway, NJ). Runs were conducted at 25 °C with HBS-EP buffer comprising 10 mM HEPES (pH 7.4), 150 mM sodium

chloride, 3 mM EDTA, 0.005% (v/v) surfactant P20 at a flow rate of 10 μ L/min. A BoNT/A binding domain (rBoNT/A-H_C) was covalently attached to the surface of the CM5 sensor chips using standard amine coupling. The CM5 chips were activated by a 7 minute injection of a mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 0.05 M N-hydroxysuccinimide; the rBoNT/A-H_C was then injected in 10 mM sodium acetate (pH 4.0) for 20 min at a flow rate of 10 μ L/min; and unreacted succinimide esters were blocked by a 7-min injection of 1 M ethanolamine hydrochloride, pH 8.5. The immobilized amount of rBoNT/A-H_C on the chip was reflected by an approximate 10,000 increase in response units (about 10 ng/mm²). Membrane extracts from SiMa, PC-12 or Neuro-2a cells transfected with FGFR1, FGFR2, FGFR3, or GFP and membrane extracts from SiMa or SH-SY5Y cells transfected with FGFR3, SV2C, FGFR3 and SV2C or empty vector (pcDNA3.1) were prepared and diluted to a final concentration of 5 μ g/mL total protein with HBS-EP buffer and added BIAcore Nonspecific Noise Reducer to a final concentration of 1 μ g/mL. The membrane analyte samples were flowed over the rBoNT/A-H_C chip surface, allowing an association time of 10 minutes and a dissociation time of 20 minutes. The surface was regenerated by two runs of 1 minute injection of 10 mM glycine-HCl (pH 1.5) at a flow rate of 30 μ L/min. The results were evaluated using BIAcore Software 3.0. As a measure for binding the maximal mass change, RU_{max} for each of the curves were recorded and the percentage increase in binding was calculated, by comparing the RU_{max} for the transfected cell lines to the cell lines transfected with GFP only.

[0203] Transfection with FGFR2c or FGFR3c increased the binding to rBoNT/A-H_C in all three cell lines tested, while transfection with FGFR1c increased the binding to rBoNT/A-H_C in PC-12 and Neuro-2a cells only (Tables 6 and 7). Since transfection with FGFR1c only increased BoNT/A uptake in PC-12 cells in the SNAP-25₁₉₇ Western blot assay, the binding to FGFR1c or internalization via FGFR1c suggests that these events are dependent on an additional factor or factors not present in some of the cells tested, and that BoNT/A binding to FGFR1c in Neuro-2a cells does not lead to BoNT/A uptake.

Table 6. Analysis of Binding of FGFR1, FGFR2, FGFR3, and GFP				
SPR Parameter	SiMa			
	FGFR1	FGFR2	FGFR3	GFP
RUMax	330	450	450	320
Percentage increase	3	40	40	
SPR Parameter	PC-12			
	FGFR1	FGFR2	FGFR3	GFP
RUMax	480	510	510	350
Percentage increase	37	46	46	
SPR Parameter	Neuro-2a			
	FGFR1	FGFR2	FGFR3	GFP
RUMax	470	460	510	370
Percentage increase	27	24	38	

Table 7. Analysis of Binding of FGFR3, SV2C, FGFR3/SV2C and Empty Vector	
SPR Parameter	SiMa

	FGFR3	SV2C	FGFR3/SV2C	Empty V
RUMax	225	140	165	130
Percentage increase	73	8	27	
SPR Parameter	SH-SY5Y			
	FGFR3	SV2C	FGFR3/SV2C	Empty V
RUMax	110	85	90	80
Percentage increase	38	6	13	

[0204] Previously, to determine the changes in genome-wide gene transcription levels in cells susceptible to BoNT/A intoxication, gene expression analysis was performed on cell lines comprising cells exhibiting various degrees of susceptibility to BoNT/A intoxication, in both differentiated and undifferentiated states using GENECHIP® U133 Plus 2 microarray technology (Affymetrix, Inc., Santa Clara, CA). See e.g., Jacky, et al., *Cells Useful for Immuno-Based BoNT/A Activity Assays*, U.S. Patent Application 12/722,801; Jacky, et al., *Cells Useful for Immuno-Based BoNT/A Activity Assays*, International Patent Application PCT/US2010/027242, each of which is hereby incorporated by reference in its entirety. This analysis showed that FGFR2 was upregulated in cells showing higher sensitivity to BoNT/A intoxication relative to cells showing lower sensitivity to BoNT/A intoxication. For example, cell lines comprising cells having high sensitivity to BoNT/A intoxication exhibited a 6-7-fold higher expression level for FGFR2 transcripts relative to a cell line comprising cells having lower sensitivity to BoNT/A intoxication. In addition, differentiated cells showed increased sensitivity BoNT/A, and this increased sensitivity was correlated with increased transcript expression of FGFR2.

[0205] To confirm the microarray expression analysis, protein levels of FGFR2 were assessed by staining cell membranes with α -FGFR2 polyclonal antibodies and analyzing the resultant staining pattern using FACS. Approximately 5×10^6 cells from a parental SiMa cell line of the SiMa H1 clonal cell line were harvested from a culture under undifferentiated or differentiated conditions, washed once with a staining buffer comprising 0.5% BSA in PBS, and then centrifugation at 1,200 rpm for 3 minutes to pellet the cells. The cell pellet was resuspended in 200 μ L of staining buffer, 5 μ L of α -FGFR2 polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) was added, and the primary antibody mixture was incubated for 1 hour at 4 °C. After primary antibody incubation, the mixture was washed by adding 10 mL of staining buffer and centrifuged at 1,200 rpm for 3 minutes to pellet the cells. The cell pellet was resuspended in 200 μ L of staining buffer, 2 μ L of goat α -rabbit IgG FITC polyclonal antibodies (Abcam, Cambridge, MA) was added and the secondary antibody mixture was incubated for 1 hour at 4 °C protected from the light. After secondary antibody incubation, the mixture was washed by adding 10 mL of staining buffer and centrifuged at 1,200 rpm for 3 minutes to pellet the cells. The cell pellet was resuspended in 500 μ L of staining buffer and samples from this mixture were analyzed using BD FACSCanto flow cytometer (BD Biosciences, San Jose, CA). Collected data was evaluated using FlowJo software (Tree Star Inc., Ashland, OR).

[0206] This analysis indicated that as the protein level of FGFR2 increases in the cell membrane, the sensitivity to BoNT/A intoxication increases (Table 8). For example, growth of cells in serum free medium

promotes differentiation and, as disclosed in the present specification, differentiation of SiMa cells increases the cell's sensitivity to BoNT/A intoxication. Similarly, the addition of ganglioside GT1b increases the sensitivity to BoNT/A intoxication in cells expressing FGFR2.

[0207] This analysis indicated that as the protein level of FGFR2 increases in the cell membrane, the sensitivity to BoNT/A intoxication increases (Table 8). For example, cells from the SiMa H1 clonal cell line were more sensitive to BoNT/A intoxication in both undifferentiated and differentiated conditions when compared to cells from the SiMa parental cells. As detailed in Table 8, cells from the SiMa H1 cell line expressed higher levels of FGFR2 under every culture condition tested. Moreover, plating of cells in serum free medium promotes differentiation and, as disclosed in the present specification, differentiation of parental SiMa or SiMa H1 cells increased the cell sensitivity to BoNT/A intoxication. Thus, there is a clear correlation between increased FGFR2 expression during cell differentiation in both cell lines with increase BoNT/A sensitivity. Similarly, the addition of ganglioside GT1b increased cell sensitivity to BoNT/A intoxication in cells expressing FGFR2.

Table 8. FACS Analysis of Cells Expressing FGFR2			
Growth Condition	Cell Count	Relative Fluorescence Intensity Value	Mean Fluorescence Intensity Value
SiMa (GM)	10,000	8.92	342
SiMa (SFM)	10,000	67.6	884
SiMa (SFM, GT1b)	10,000	257	1291
SiMa H1 (GM)	10,000	97.2	919
SiMa H1 (SFM)	10,000	160	2526
GM, Growth Medium; SFM, Serum Free Medium; GT1b, ganglioside GT1b			

Example XI

SV2 Enhances BoNT/A Binding to FGFR3

[0208] To determine the role of SV2 in BoNT/A binding to FGFR2 or FGFR3, the binding of BoNT/A to FGFR3 and/or SV2 was characterized using Surface Plasmon Resonance analysis. Runs were conducted at 25 °C with HBS-EP buffer comprising 10 mM HEPES (pH 7.4), 150 mM sodium chloride, 3 mM EDTA, 0.005% (v/v) surfactant P20 at a flow rate of 10 µL/min. The BoNT/A binding domain (rBoNT/A-H_C) or the protease inactive version of BoNT/A, iBoNT/A were covalently attached to the surface of the CM5 sensor chip using standard amine coupling. The CM5 chip were activated by a 7 minute injection of a mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 0.05 M N-hydroxysuccinimide; the rBoNT/A-H_C or iBoNT/A was then injected in 10 mM sodium acetate (pH 4.0) for 20 min at a flow rate of 10 µL/min; and unreacted succinimide esters were blocked by a 7-min injection of 1 M ethanolamine hydrochloride, pH 8.5. The immobilized amount of rBoNT/A-H_C or iBoNT/A on the chip was reflected by an 500-1000 increase in response units (0.5-1 ng/mm²). From 250 nM to 35 µM of sFGFR3 or SV2C₅₂₉₋₅₆₆ were flowed over the rBoNT/A-H_C or iBoNT/A chip surface, allowing an association time of 10 minutes and a dissociation time of 20 minutes. The surface was regenerated by

one run of 1 minute injection of 10 mM glycine-HCl (pH 1.5) at a flow rate of 30 μ L/min. The results were evaluated using BIAcore Software 3.0 and the KD for each of the peptides upon binding to either rBoNT/A-Hc or iBoNT/A was calculated (Table 9). High affinity binding was observed between rBoNT/A-Hc or iBoNT/A and loop 2 and 3 of the extra cellular domain of FGFR3, sFGFR3 (KD about 25 nM and about 10 nM respectively). In comparison, no binding was observed between rBoNT/A-Hc and the luminal part of SV2C, SV2C₅₂₉₋₅₆₆ and only weak binding was observed between iBoNT/A and SV2C₅₂₉₋₅₆₆ (KD about 5 μ M). However, when SV2C₅₂₉₋₅₆₆ was combined with sFGFR3 the binding of sFGFR3 to rBoNT/A-Hc was enhanced (KD about 1 nM). In addition, a BIAcore chip surface covered with immobilized rBoNT/A-Hc could recover FGFR3 from cell membrane extracts.

TABLE 9. BIAcore Analysis of Binding of FGFR3 and SV2C to BoNT/A

Immobilized protein	Analyte	KD
BoNT/A-Hc	sFGFR3	25 nM
BoNT/A-Hc	SV2C ₅₂₉₋₅₆₆	No binding
BoNT/A-Hc	sFGFR3/ SV2C ₅₂₉₋₅₆₆	1 nM
iBoNT/A	sFGFR3	10 nM
iBoNT/A	SV2C ₅₂₉₋₅₆₆	5 μ M
iBoNT/A	sFGFR3/ SV2C ₅₂₉₋₅₆₆	—

[0209] In closing, it is to be understood that although aspects of the present specification have been described with reference to the various embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of the principles of the subject matter disclosed in the present specification. Therefore, it should be understood that the disclosed subject matter is in no way limited to a particular methodology, protocol, and/or reagent, etc., described herein. As such, various modifications or changes to or alternative configurations of the disclosed subject matter can be made in accordance with the teachings herein without departing from the spirit of the present specification. Lastly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Accordingly, the present invention is not limited to that precisely as shown and described.

[0210] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0211] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0212] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” As used herein, the term “about” when qualifying a value of a stated item, number, percentage, parameter, or term refers to a range of plus or minus ten percent of the value of the stated item, number, percentage, parameter, or term. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0213] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0214] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term “consisting of” excludes any element, step, or ingredient not specified in the claims. The transition term “consisting essentially of” limits the scope of a claim to the specified materials or

steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

[0215] All patents, patent publications, and other publications referenced and identified in the present specification are individually and expressly incorporated herein by reference in their entirety for the purpose of describing and disclosing, *e.g.*, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

CLAIMS

What is claimed:

1. A method of detecting BoNT/A activity by contacting a sample to a cell that contains an exogenous FGFR2 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.
2. The method according to 1, wherein said FGFR2 is a mammalian FGFR2.
3. The method according to Claim 1, wherein the cell further comprises a FGFR3.
4. The method according to 3, wherein said FGFR3 is a mammalian FGFR3.
5. The method according to Claim 1, wherein the cell further comprises a SV2.
6. The method according to Claim 3, wherein said SV2 is a SV2A, a SV2B, or a SV2C.
7. The method according to Claim 1, wherein said cell further contains a polysialoganglioside.
8. The method according to Claim 4, wherein said polysialoganglioside is selected from the group consisting of GD1a, GD1b, GD3, GQ1b, or GT1b.
9. A method of detecting BoNT/A activity by contacting a sample to a cell that contains an endogenous FGFR2 and an exogenous FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.
10. The method according to Claim 9, wherein the cell further comprises a SV2.
11. The method according to Claim 9, wherein said cell further contains a polysialoganglioside.
12. A method of detecting BoNT/A activity by contacting a sample to a cell that contains an exogenous FGFR2 and an endogenous FGFR3 wherein said contacted cell is capable of BoNT/A intoxication and detecting the presence of BoNT/A activity of said contacted cell relative to a control cell, where a difference in said BoNT/A activity of said contacted cell as compared to said control cell is indicative of BoNT/A activity.

13. The method according to Claim 9, wherein the cell further comprises a SV2.
14. The method according to Claim 9, wherein said cell further contains a polysialoganglioside.

FIG. 1.

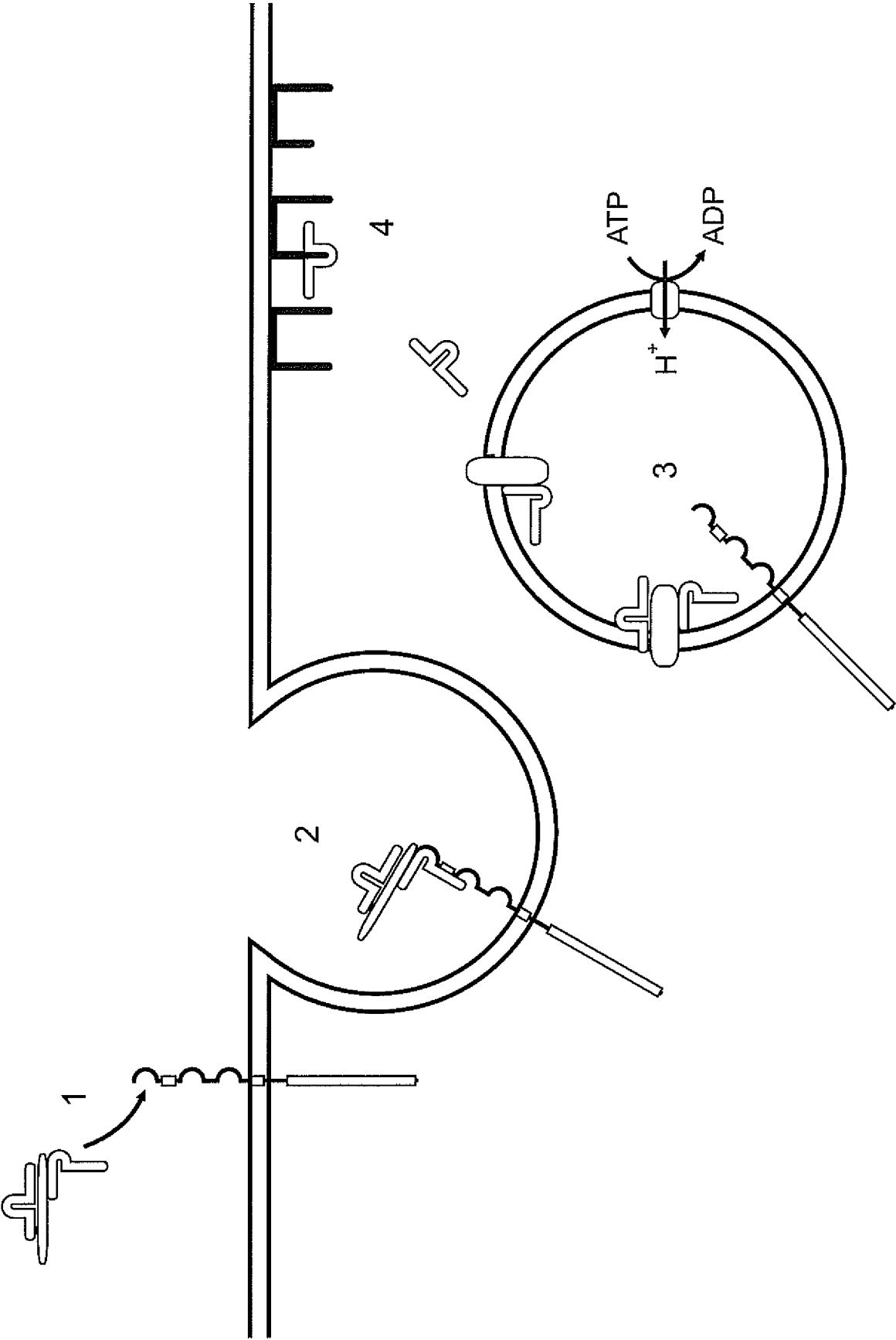


FIG. 2.

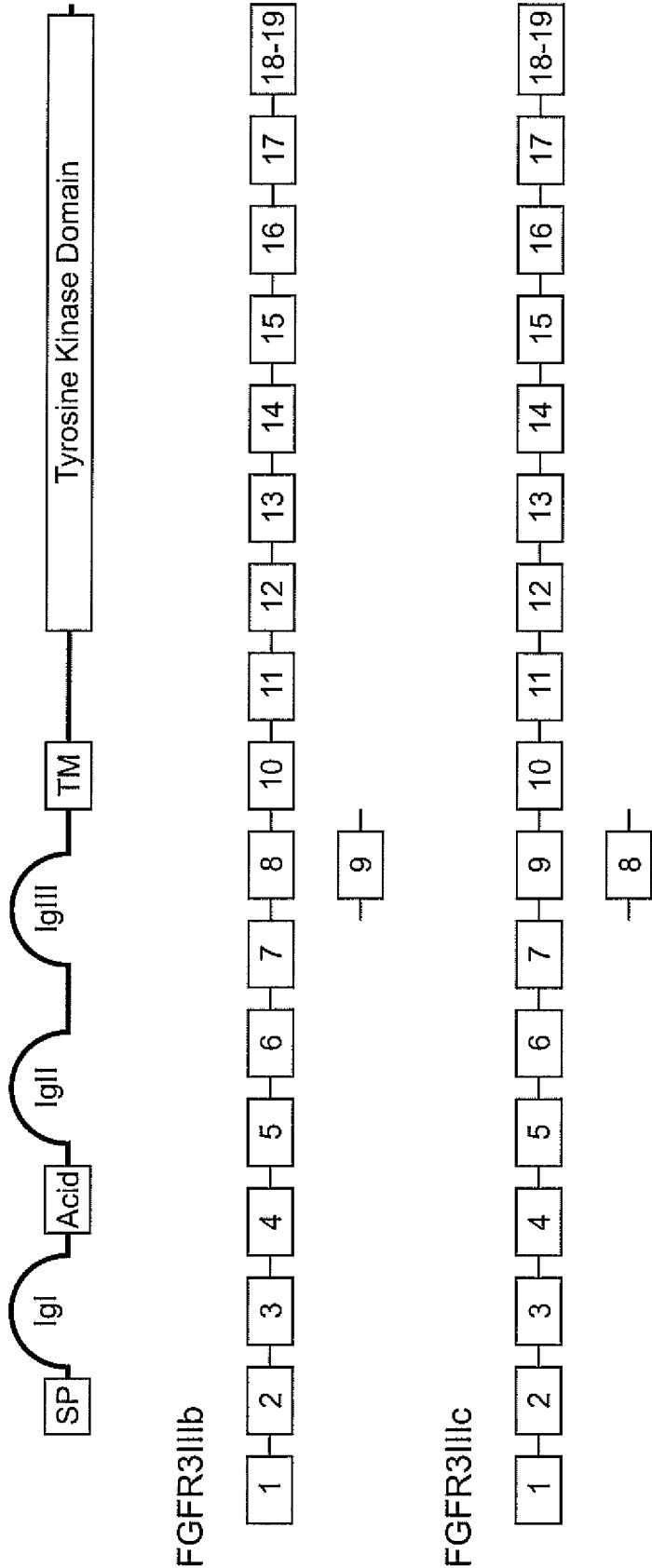


FIG. 3a.

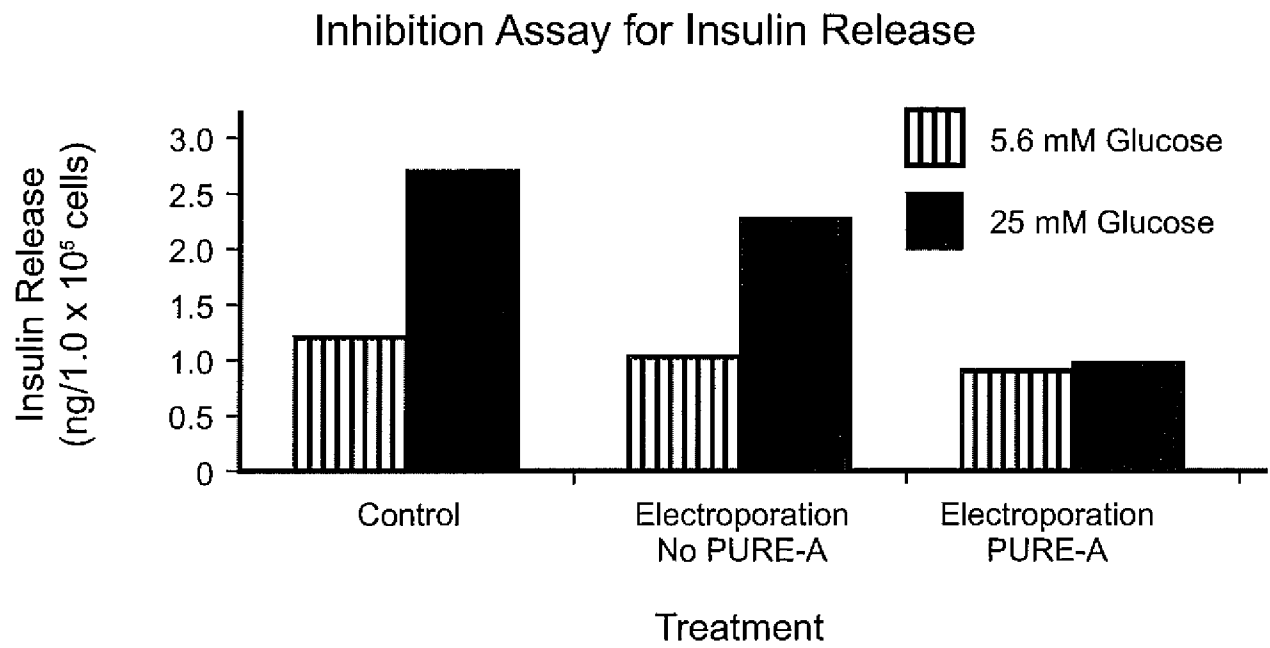


FIG. 3b.

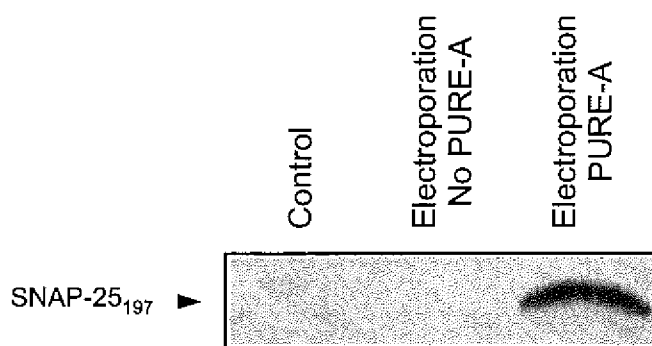


FIG. 4a.

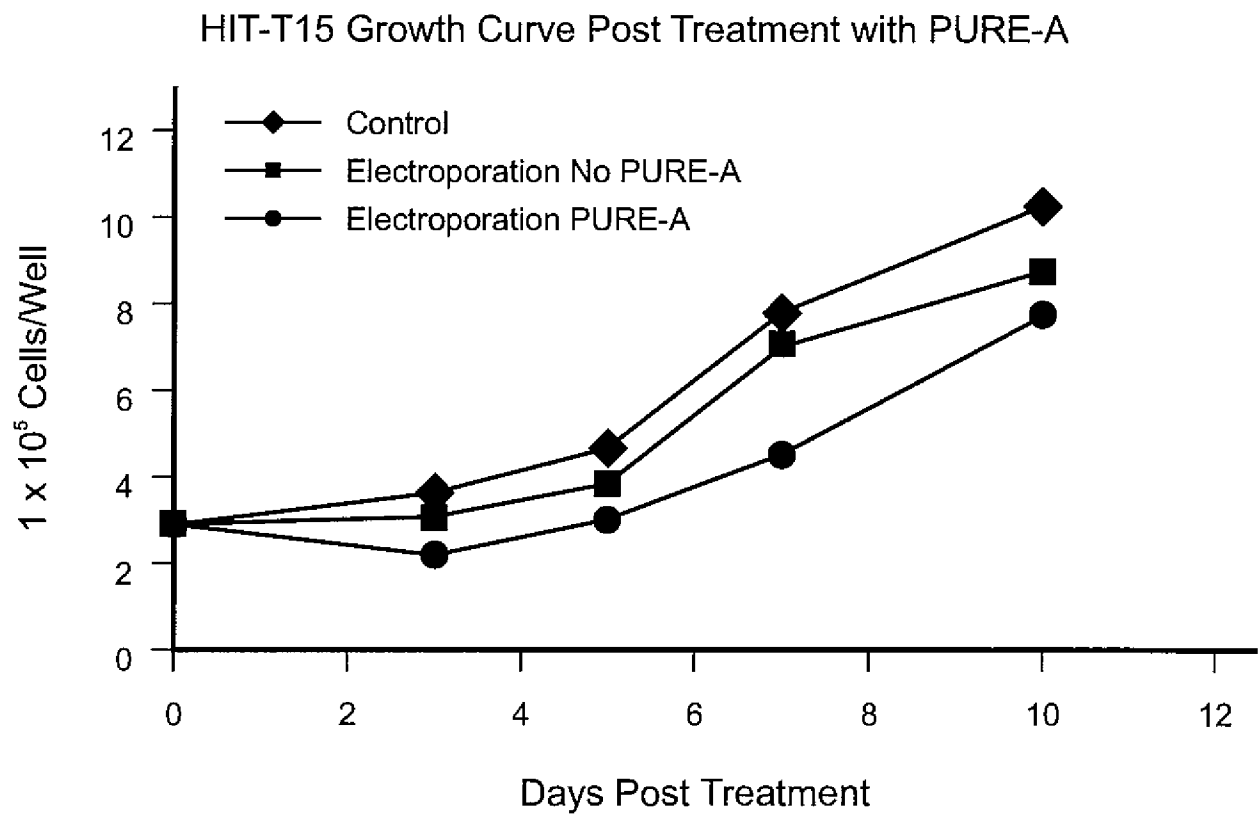


FIG. 4b.

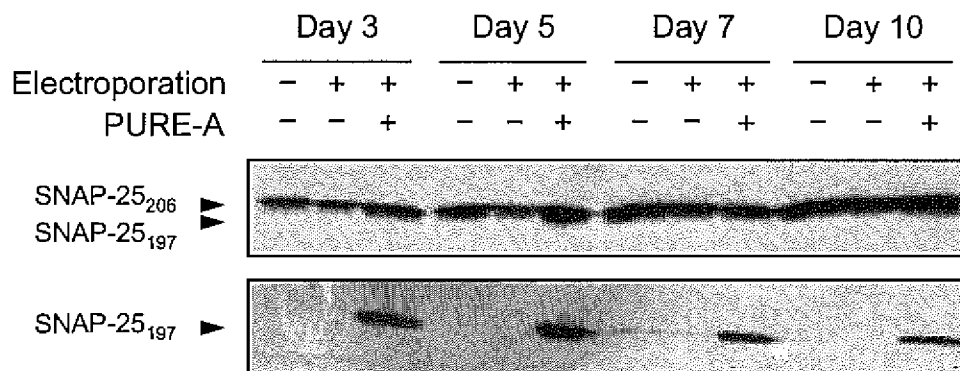


FIG. 5.

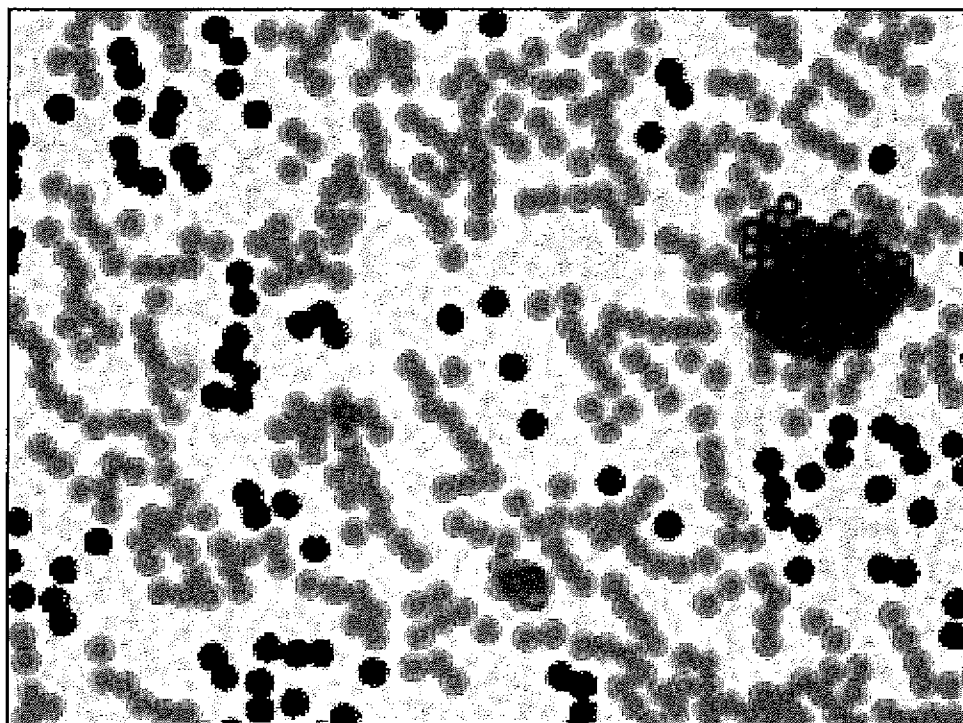


FIG. 6.

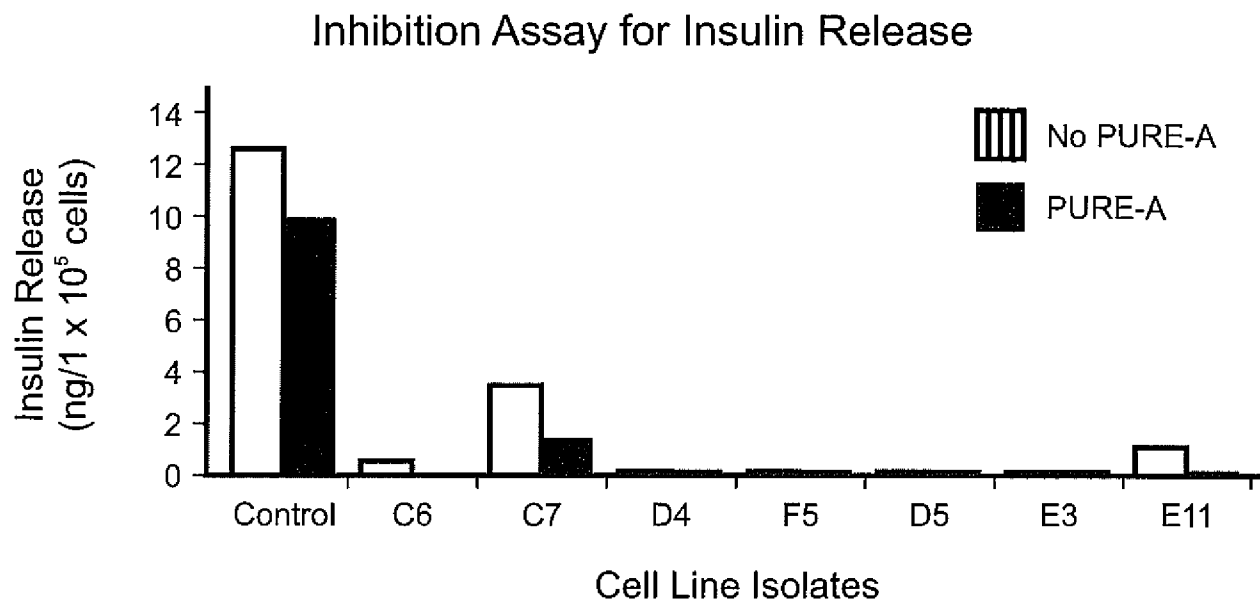


FIG. 7a.

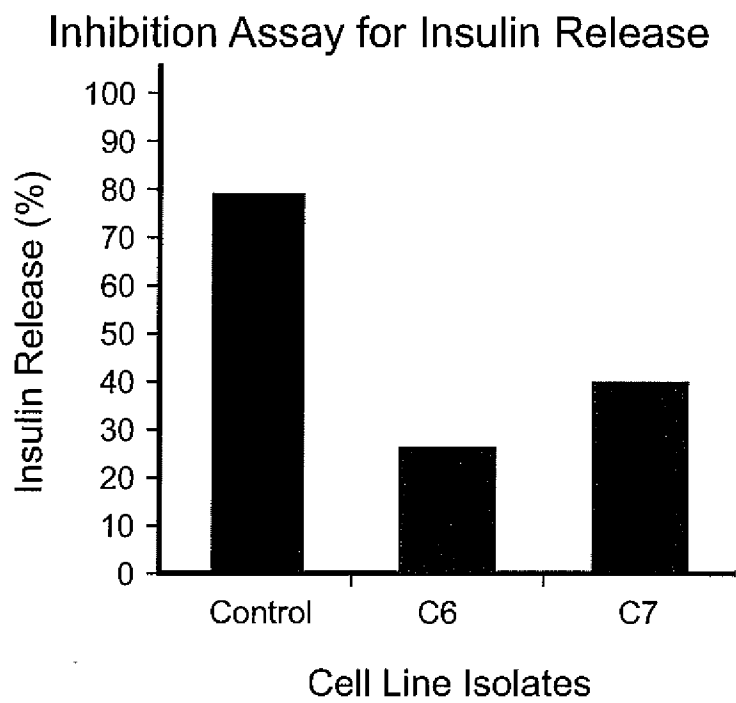


FIG. 7b.

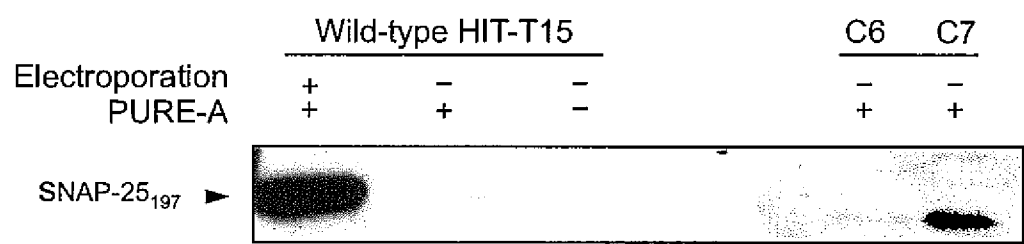


FIG. 8a.

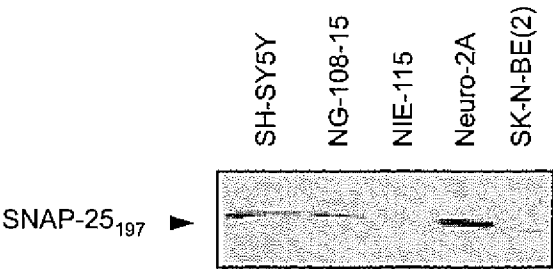


FIG. 8b.

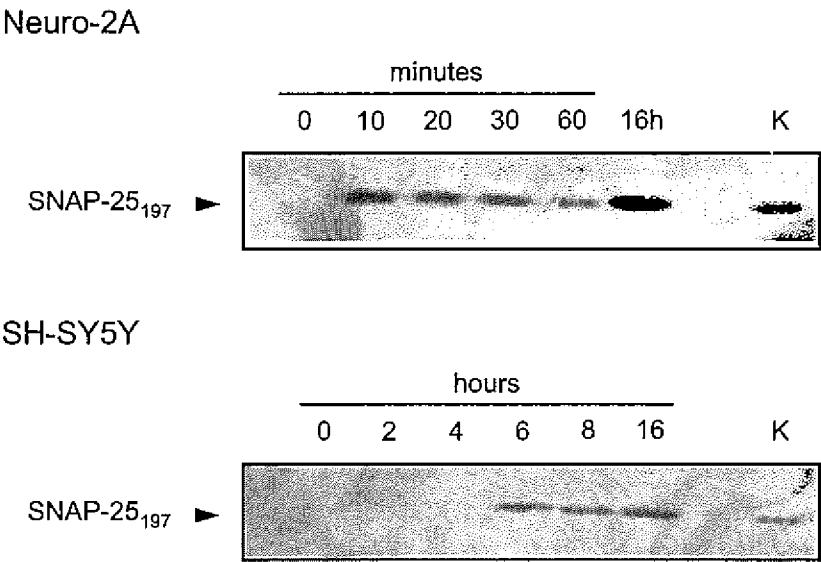


FIG. 8c.

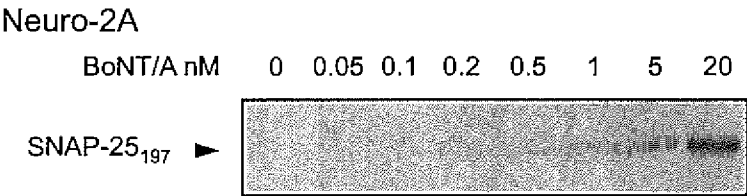


FIG. 9a.

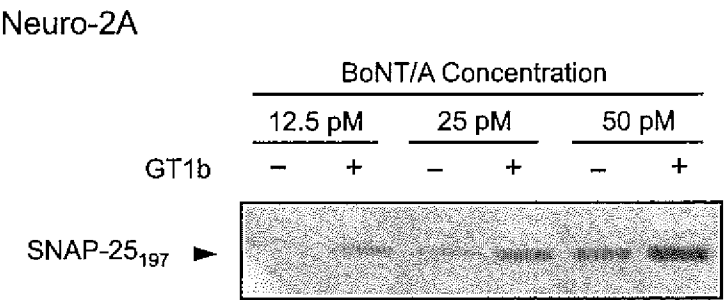


FIG. 9b.

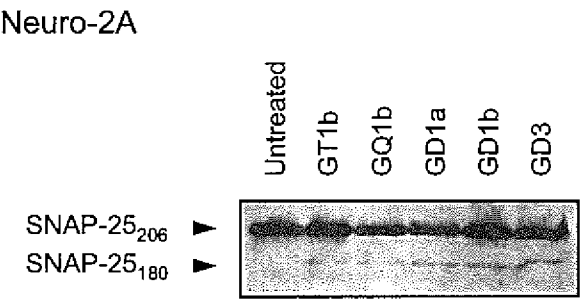


FIG. 10a.

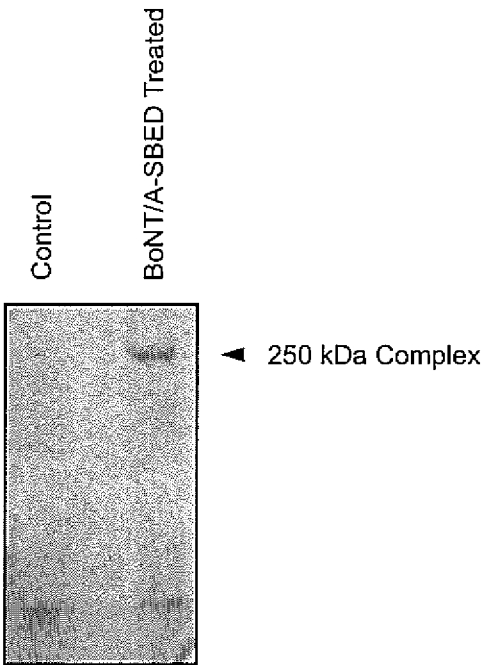


FIG. 10b.

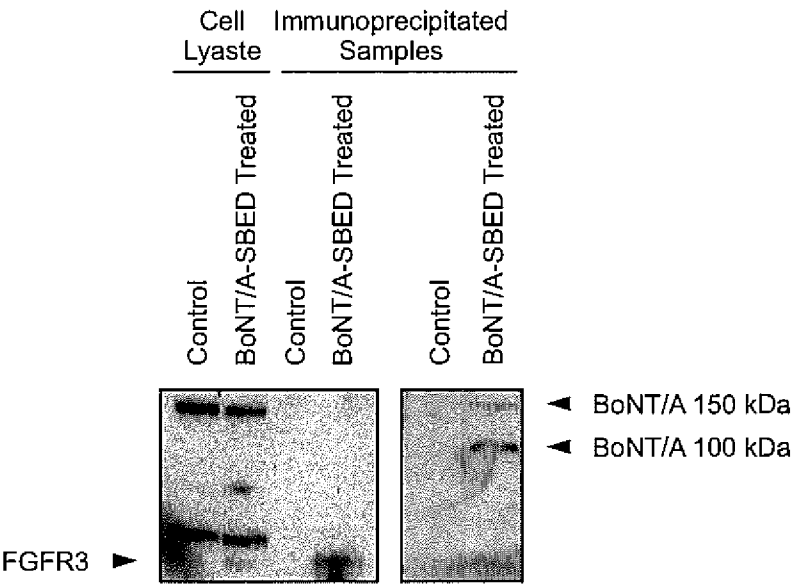


FIG. 11.

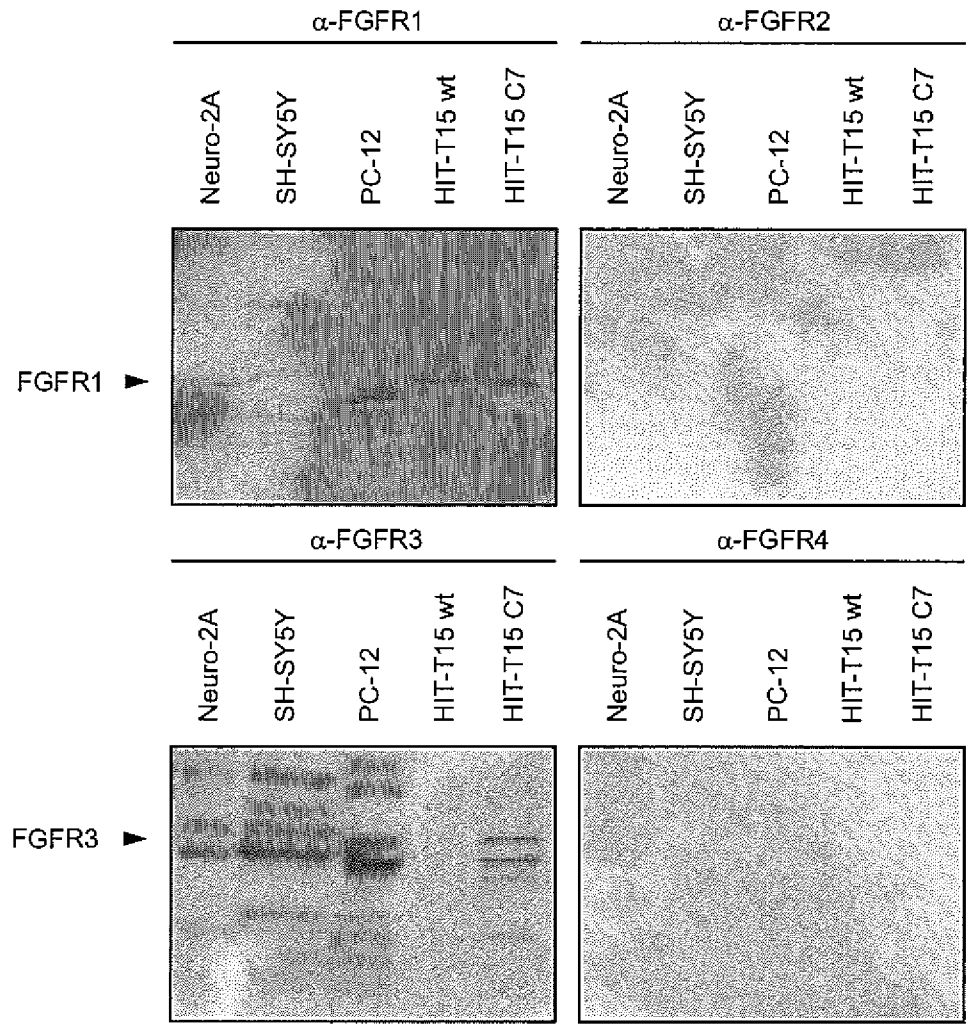


FIG. 12a.

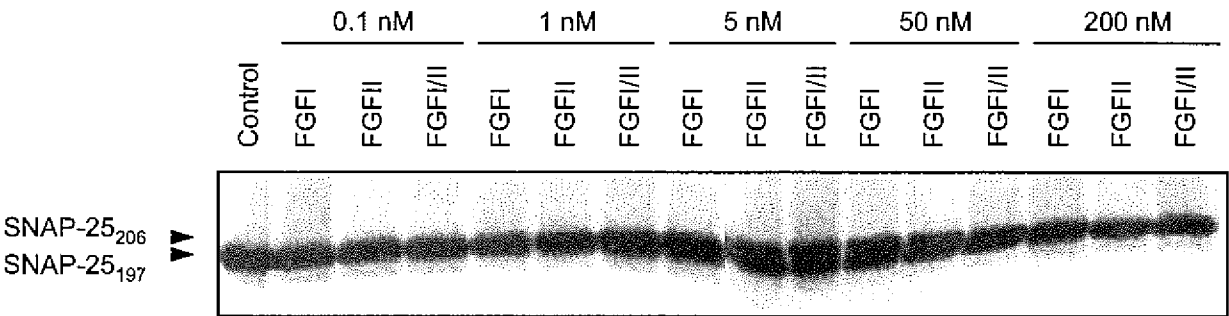


FIG. 12b.

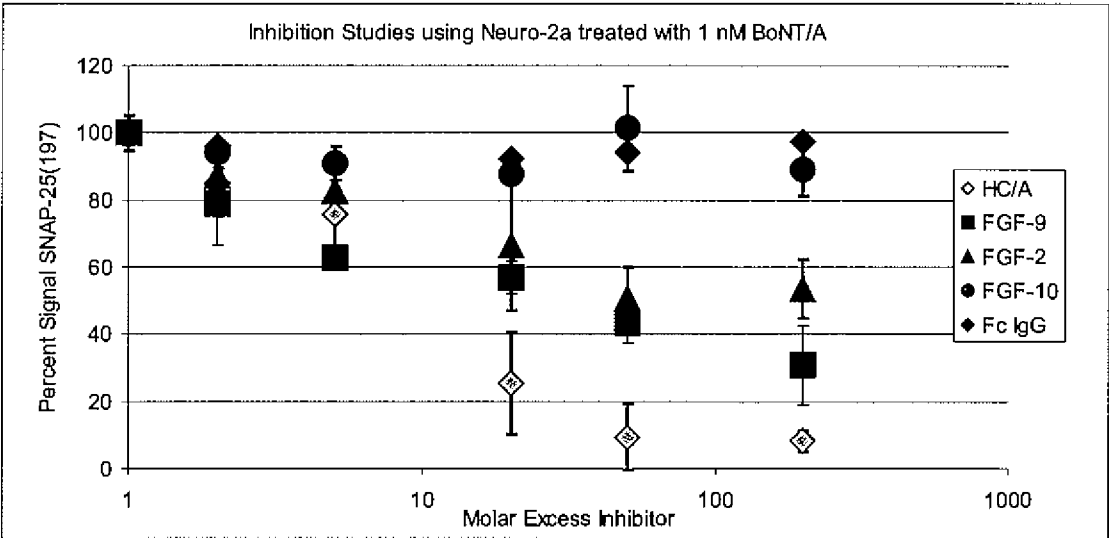


FIG. 13a.

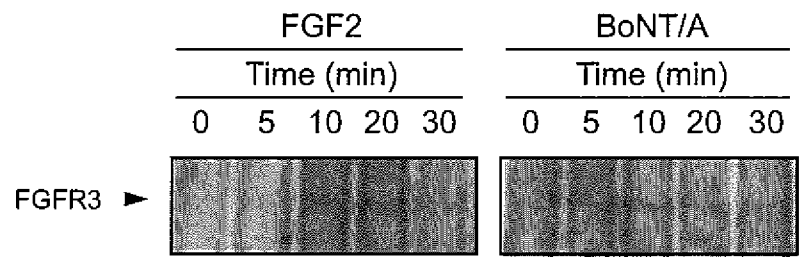


FIG. 13b.

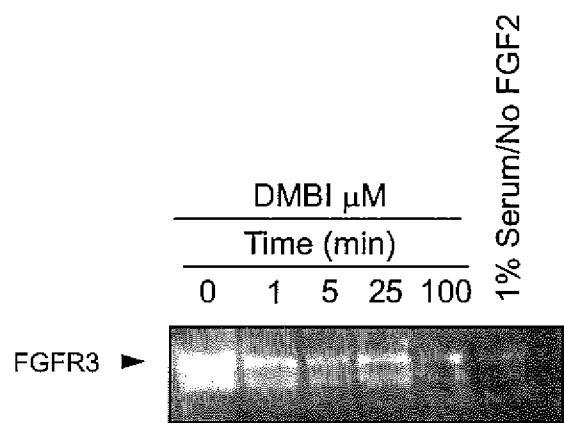
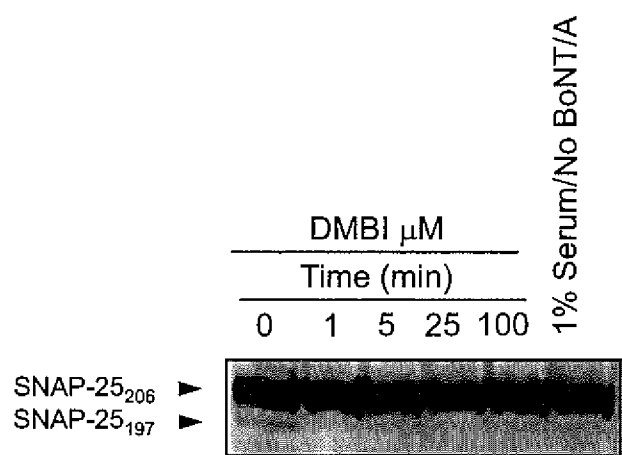


FIG. 13c.



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/035583

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50 G01N33/569

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)

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 practical, search terms used)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ESTER F S ET AL: "Identification of the fibroblast growth factor receptor FGFR3 as a component of the receptor complex for Botulinum Neurotoxin Type A" TOXICON, ELMSFORD, NY, US LNKD-DOI:10.1016/J.TOXICON.2008.04.008, vol. 51, 1 June 2008 (2008-06-01), page 3, XP022695696 ISSN: 0041-0101 [retrieved on 2008-05-29] the whole document	1-14
X	US 2008/182799 A1 (FERNANDEZ-SALAS ESTER [US] ET AL) 31 July 2008 (2008-07-31) claims 1-26 paragraph [0032]; table 1 examples 1-10	1-14

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

7 July 2010

Date of mailing of the international search report

15/07/2010

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/035583

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO 2009/114748 A1 (ALLERGAN INC [US]; FERNANDEZ-SALAS ESTER [US]; WANG JOANNE [US]; GARAY) 17 September 2009 (2009-09-17) paragraph [0117] - paragraph [0119] paragraph [0157] -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/035583

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		CA 2558758 A1	09-09-2005
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		JP 2007526770 T	20-09-2007
		US 2009317839 A1	24-12-2009
		US 2008003240 A1	03-01-2008
		WO 2005082096 A2	09-09-2005
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