[0004] While the general mechanism of action (MoA) for BoNT/A at the presynaptic nerve terminal is well established (Montal, M., (2010). "Botulinum neurotoxin: a marvel of protein design." *Annu.Rev.Biochem.* 79, 591-617.), there are still many unanswered questions regarding the intracellular trafficking patterns and general 'life-cycle' of the toxin. Resolving these questions partly depends on the ability to precisely detect the toxin's location, distribution, and movement within a cell. Direct detection of BoNT/A using antibodies is difficult due to its high potency and therefore, extremely low concentration within neurons. An alternative approach for detecting the presence of BoNT/A has been to track its enzymatic activity via immuno-staining for the cleaved SNAP25 product ($SNAP25_{197}$). Both commercial and proprietary antibodies have been used to trace the expression of full-length SNAP25 ($SNAP25_{206}$) or BoNT/A-cleaved SNAP25 ($SNAP25_{197}$). However, the terminal epitope of $SNAP25_{197}$ that is generated following BoNT/A cleavage is difficult to specifically target with an antibody without also recognizing the intact SNAP25 protein (Mort, J.S. and Buttle, D.J., (1999). "The use of cleavage site specific antibodies to delineate protein processing and breakdown pathways." *Mol.Pathol.* 52, 11-18.). Consequently, immuno-staining results have been misleading, with some antibodies being assay-dependent while other antibodies are tissue-specific. There is therefore a need for a very selective antibody against BoNT/A-cleaved SNAP25 that can identify $SNAP25_{197}$ in any tissue-type and in multiple assays following exposure to BoNT/A.

[0005] The present disclosure addresses these issues by providing methods and compositions for detecting botulinum toxin cleaved SNAP25, including BoNT/A cleaved SNAP25 and any other BoNT/A-related compounds that cleave SNAP25 at position '197', in various different assays, such as but not limited to immunohistochemistry using highly specific recombinant monoclonal antibodies (rMAb) against $SNAP25_{197}$. These antibodies can be used to detect $SNAP25_{197}$ in a variety of tissues from different species, such as but not limited to human. These antibodies can also be used as tools to diagnose activity and efficacy in tissues from humans that have been treated with neurotoxin, such as but not limited to onabotulinumtoxinA.

SUMMARY OF THE INVENTION

[0006] The invention encompasses an anti-SNAP25 antibody wherein the antibody binds preferentially to a BoNT/A cleaved SNAP25.

[0006a] In one aspect, the invention encompasses an anti-SNAP25 antibody wherein the antibody binds preferentially to BoNT/A cleaved SNAP25, wherein the antibody does not

bind to full length SNAP25, and is characterized in that the antibody is a recombinant murine antibody and comprises a light chain sequence of SEQ ID NO:1 and a heavy chain sequence of SEQ ID NO:3, or wherein the antibody is a recombinant human antibody and comprises a light chain sequence of SEQ ID NO:5 and a heavy chain sequence of SEQ ID NO:7

[0006b] In some embodiments, the anti-SNAP25 antibody binds preferentially to a SNAP25 that has been cleaved by a recombinant botulinum

toxin with enzymatic (light chain) activity of botulinum toxin serotype A. In other embodiments, the anti-SNAP25 antibody does not bind to full length or uncleaved SNAP25.

[0007] In other embodiments, the anti-SNAP25 antibody is able to detect BoNT/A cleaved SNAP25 (or SNAP25 cleaved by a recombinant botulinum toxin with enzymatic (light chain) activity of botulinum toxin serotype A) in a tissue. In some embodiments, the tissue is a biopsy sample. In other embodiments, the tissue is a skin punch.

[0008] In some embodiments, the anti-SNAP25 antibody comprises a light chain sequence of SEQID NO:1 or SEQID NO: 2 and is a recombinant murine antibody. In other embodiments, the anti-SNAP25 antibody comprises a heavy chain sequence of SEQID NO:3 or SEQID NO:4 and is a recombinant murine antibody. In other embodiments, the anti-SNAP25 antibody comprises a light chain sequence of SEQID NO:5 or SEQID NO: 6 and is a recombinant human antibody. In other embodiments, the anti-SNAP25 antibody comprises a heavy chain of SEQID NO:7 or SEQID NO:8 and is a recombinant human antibody. In other embodiments, the anti-SNAP25 antibody is an antibody that binds to the same epitope of an antibody with a heavy chain and/or light chain comprising one or more sequences of SEQID NOs:1-8.

[0008a] In one aspect, the invention encompasses a method of diagnosing if a tissue has been exposed to BoNT/A enzymatic activity comprising contacting a tissue sample suspected of having been exposed to BoNT/A enzymatic activity with an anti-SNAP25 antibody wherein the antibody binds preferentially to BoNT/A cleaved SNAP25 and is defined herein; and detecting whether the anti-SNAP25 antibody bound to the tissue sample, wherein the presence of the anti-SNAP25 antibody binding to the tissue sample indicates that the tissue sample has been exposed to BoNT/A activity.

[0009] The invention also encompasses a method of diagnosing if a tissue has been exposed to BoNT/A enzymatic activity comprising contacting a tissue sample suspected of having been exposed to BoNT/A enzymatic activity with an anti-SNAP25 antibody wherein the antibody preferentially binds to a BoNT/A cleaved SNAP25; and detecting whether the anti-SNAP25 antibody bound to the tissue sample, wherein the presence of the anti-SNAP25 antibody binding to the tissue sample indicates that the tissue sample has been exposed to BoNT/A enzymatic activity. In some embodiments, the BoNT/A enzymatic activity is from a native BoNT/A. In other embodiments, the BoNT/A activity is from a recombinant botulinum toxin with enzymatic (light chain) activity of botulinum toxin serotype A. In some embodiments

the anti-SNAP25 antibody used in the method comprises a light chain sequence of SEQID NO:1 or SEQID NO: 2 and is a recombinant murine antibody. In other embodiments, the anti-SNAP25 antibody used in the method comprises a heavy chain sequence of SEQID NO:3 or SEQID NO:4 and is a recombinant murine antibody. In other embodiments, the anti-SNAP25 antibody used in the method comprises a light chain sequence of SEQID NO:5 or SEQID NO: 6 and is a recombinant human antibody. In other embodiments, the anti-SNAP25 antibody used in the method comprises a heavy chain of SEQID NO:7 or SEQID NO:8 and is a recombinant human antibody. In other embodiments

the anti-SNAP25 antibody used in the method is an antibody that binds to the same epitope of an antibody with a heavy chain and/or light chain comprising one or more sequences of SEQID NOs:1-8.

[0009a] In another aspect, the invention encompasses a kit for diagnosing if a tissue has been exposed to BoNT/A enzymatic activity comprising an anti-SNAP25 antibody as defined herein, wherein the antibody binds preferentially to BoNT/A cleaved SNAP25.

[0010] In another aspect, the invention encompasses a kit for diagnosing if a tissue has been exposed to BoNT/A enzymatic activity comprising an anti-SNAP25 antibody, wherein the antibody binds preferentially to BoNT/A cleaved SNAP25. In some embodiments, the BoNT/A enzymatic activity is from a native BoNT/A. In other embodiments, the BoNT/A activity is from a recombinant botulinum toxin with enzymatic (light chain) activity of botulinum toxin serotype A. In some embodiments, the anti-SNAP25 antibody used in the kit comprises a light chain sequence of SEQID NO:1 or SEQID NO: 2 and is a recombinant murine antibody. In other embodiments, the anti-SNAP25 antibody used in the kit comprises a heavy chain sequence of SEQID NO:3 or SEQID NO:4 and is a recombinant murine antibody. In other embodiments, the anti-SNAP25 antibody used in the kit comprises a light chain sequence of SEQID NO:5 or SEQID NO: 6 and is a recombinant human antibody. In other embodiments, the anti-SNAP25 antibody used in the kit comprises a heavy chain of SEQID NO:7 or SEQID NO:8 and is a recombinant human antibody. In other embodiments, the anti-SNAP25 antibody used in the kit is an antibody that binds to the same epitope of an antibody with a heavy chain and/or light chain comprising one or more sequences of SEQID NOs:1-8.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 shows a diagrammatic representation of the putative epitope for the humanized and murine recombinant monoclonal antibodies against $SNAP25_{197}$. The diagram shows the 12-residue peptide, minus the N-terminal cysteine residue (used for conjugating to Keyhole Limpet Hemocyanin) that was used to generate the original monoclonal antibody .

[0012] Figure 2 (A-F) shows Western Blot analysis comparing the specificity of different antibodies for detecting full-length $SNAP25_{206}$ and cleaved $SNAP25_{197}$ in rat embryonic cortical cell lysates treated with (L_3) or without (L_2) BoNT/A at 3 nM concentration. (A) Blot probed with a commercially available anti-SNAP25 mAb (SMI-81R) that recognizes both the full-length (206) and cleaved (197) forms of SNAP25. In lane 2, only $SNAP25_{206}$ is detected, whereas in lane 3, both $SNAP25_{206}$ (arrow) and $SNAP25_{197}$ (arrowhead) are detected. (B) Blot probed with a commercially available anti-SNAP25 mAb (MC-6050) that reportedly recognizes both $SNAP25_{206}$ and $SNAP25_{197}$. Only $SNAP25_{197}$

WE CLAIM:

1. An anti-SNAP25 antibody wherein the antibody binds preferentially to BoNT/A cleaved SNAP25,
wherein the antibody does not bind to full length SNAP25, and is characterized in that the antibody is a recombinant murine antibody and comprises a light chain sequence of SEQ ID NO:1 and
a heavy chain sequence of SEQ ID NO:3, or
wherein the antibody is a recombinant human antibody and comprises a light chain sequence of SEQ ID NO:5 and
a heavy chain sequence of SEQ ID NO:7.

2. The anti-SNAP25 antibody of claim1 wherein the antibody is able to preferentially bind to BoNT/A cleaved SNAP25 in a tissue.

3. The anti-SNAP25 antibody of claim 2, wherein the tissue is a biopsy sample.

4. A method of diagnosing if a tissue has been exposed to BoNT/A enzymatic activity comprising contacting a tissue sample suspected of having been exposed to BoNT/A enzymatic activity with an anti-SNAP25 antibody wherein the antibody binds preferentially to BoNT/A cleaved SNAP25 and is defined according to any one of claims 1 – 3 above; and detecting whether the anti-SNAP25 antibody bound to the tissue sample, wherein the presence of the anti-SNAP25 antibody binding to the tissue sample indicates that the tissue sample has been exposed to BoNT/A activity.

5. A kit for diagnosing if a tissue has been exposed to BoNT/A enzymatic activity comprising an anti-SNAP25 antibody as defined in any one of claims 1 – 3 above, wherein the antibody binds preferentially to BoNT/A cleaved SNAP25.