Antibody Validation as a tool to study “Synaptic Adhesion Proteins”

by

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Abstract

Historically there has been an absence of reliable and validated antibodies against synaptic adhesion proteins, particularly neurexins and neuroligins. This has led to the failure of the vital experiments that study the function of these synaptic proteins, leading to wastage of time and expensive resources. My project validated antibodies against neurexins and neuroligins through the process of immunoprecipitation, which pulls the protein of interest, followed by the process of western blot to visualize the results. The results indicated that the antibodies against β isoforms across the three neurexins performed better. Similarly, antibodies against Nlgn 2 and Nlgn 3 showed consistent bands congruent with the molecular weight of neuroligin. This project also investigated the role of Nrxn1γ, an isoform of neurexin1, in maintaining the excitatory-inhibitory balance between synapses that is required for optimal synaptic function. Collectively, these findings not only establish a dependable set of antibodies for probing neurexin and neuroligin biology but also underscore Nrxn1γ as a meaningful contributor to inhibitory synapse maintenance, strengthening both the technical and conceptual foundations for future synaptic research.

Acknowledgements

I would like to offer my deepest gratitude to God Almighty for granting me perseverance and strength throughout this journey.

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I am deeply thankful to my parents and family, who supported me through restless nights and eased my anxiety with their calmness and advice.

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Introduction

The human brain is composed of billions of neurons distributed across diverse functionally specialized regions (Colón-Ramos 2009). Neurons form intricate networks through specialized junctions known as synapses, which serve as the fundamental sites for processing and transmitting information between cells (Siddiqui and Craig 2010; Südhof 2018). Changes in synaptic strength, called synaptic plasticity, allow neural circuits to adapt to experience, and this flexibility is the biological basis for learning, memory, and other cognitive functions (Goda and Sabatini 2011; Ramirez and Arbuckle 2016). An example of experience-dependent synaptic plasticity is addiction, where an increase in synaptic strength induces substance craving and decrease in synaptic strength can subdue substance craving (Ramirez and Arbuckle 2016). Synapses are broadly classified as excitatory or inhibitory and their balanced interplay, known as excitation-inhibition balance, is essential for maintaining network stability and supporting cognitive function. For example, mice with E/I imbalance show social deficits, such as repetitive behaivours, that are congruent with behaivours associated with autism spectrum disorder (ASD) (Dhume 2021; Kim 2009; Lee et al. 2016).

Synaptic junctions are organized by synaptic adhesion molecules (SAMs), a diverse class of cell-adhesion proteins that mediate trans-synaptic interactions and coordinate the differentiation of pre- and postsynaptic compartments (Missler et al. 2012; Rudenko 2017). SAMs function to align synaptic machinery for neurotransmitter release, which involves clustering synaptic vesicles, docking proteins, and voltage-gated calcium channels on the presynaptic side (Bittmann et al. 2025). Their function on the post-synaptic side is to recruit scaffolding proteins such as PSD-95 or gephyrin, by binding to them (Rudenko 2017).

Synaptic adhesion proteins also recruit neurotransmitter receptors such as AMPA (Alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid) and NMDA (N-methy l-D-aspartate**)** receptors to the postsynaptic membrane through as yet unknown mechanisms (Rudenko 2017).

In addition to mediating synapse formation, SAMs contribute to synaptic specificity, maturation, and plasticity, thereby shaping both the structural and functional properties of neural circuits (Zhou 2023).

The most well-characterized SAMs are *neurexin*s and *neuroligin*s (Siddiqui and Craig 2010; Südhof 2017). *Neurexin*s are presynaptic cell adhesion molecules that facilitate synaptic connectivity by forming trans-synaptic bridges with their postsynaptic partners such as neuroligins (Südhof 2017). Neurexins are encoded by three genes (*Nrxn1, Nrxn2, and Nrxn3*), and each gene produces a longer α form and the shorter β form by two different promoters (Han and Kim 2007; Rudenko 2019). In general, α-neurexins are more abundant and contain six laminin-neurexin-sex hormone-binding globulin (LNS) domains interspersed with three epidermal growth factor (EGF)-like domains in their extracellular region (Fig.1) (Craig and Kang 2007). In contrast, β-neurexins possess a single LNS domain that is identical to the sixth LNS domain of the α-neurexin (Rudenko 2019). Both isoforms terminate with a PDZ-binding motif (Fig. 1) (Han and Kim 2007). The PDZ-binding motif is vital for transporting neurexins through the secretory pathway, enabling them to interact with binding partners along this route (Fairless et al. 2008). Additionally, the *Nrxn1* gene produces a third isoform, Neurexin1γ, which lacks the LNS and EGF domains present in the α and β neurexins (Fig. 1) (Connor and Siddiqui 2023; Noborn and Sterky 2021). However, the specific functions of Neurexin1γ remain largely unexplored. All neurexins have

conserved splice sites, six for α-neurexins and two for β-neurexins (Noborn and Sterky 2021). Alternative splicing at these sites generates thousands of isoforms, each contributing to distinct and specialized roles within the brain (Connor and Siddiqui 2023).

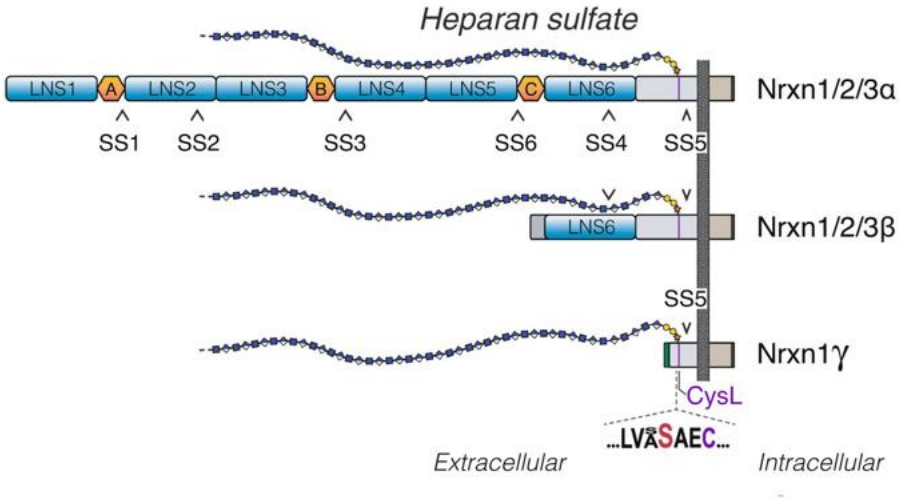

Figure 1. Schematic diagram of Neurexin structure and isoforms. LNS: laminin-neurexin-sex hormone-binding globulin. EGF: epidermal growth factor. A, B, and C: ECF domains. Arrows indicate the sites of alternative splicing. A glycosylated region (CH domain) near transmembrane domain contains site for heparan sulphate modification. Figure taken from Noborn and Sterky (2021).

Neuroligins are postsynaptic cell adhesion molecules that facilitate synaptic connections and plasticity through their interactions with presynaptic neurexins (Fig 2) (Liu et al. 2022). They are encoded by four genes: *Nlgn1*, *Nlgn2*, *Nlgn3*, and *Nlgn4*. Among these, *Nlgn1*, *Nlgn2*, and *Nlgn3* are highly conserved across species, whereas *Nlgn4* exhibits variable expression and is minimally expressed in mice (Südhof 2017). Neuroligins possess an extracellular acetylcholinesterase (AChE)-homologous domain with one or two splice site(s) depending on the neuroligin (Craig and Kang 2007). These splice sites resemble those found in neurexins, enabling the generation of numerous isoforms (Craig and Kang 2007).

Different neuroligin isoforms are expressed in different synapses (Südhof 2017). For example, Neuroligin 1 is expressed at excitatory synapses, Neuroligin 2 at inhibitory synapses and Neuroligin 3 at both (Bottos et al. 2011).

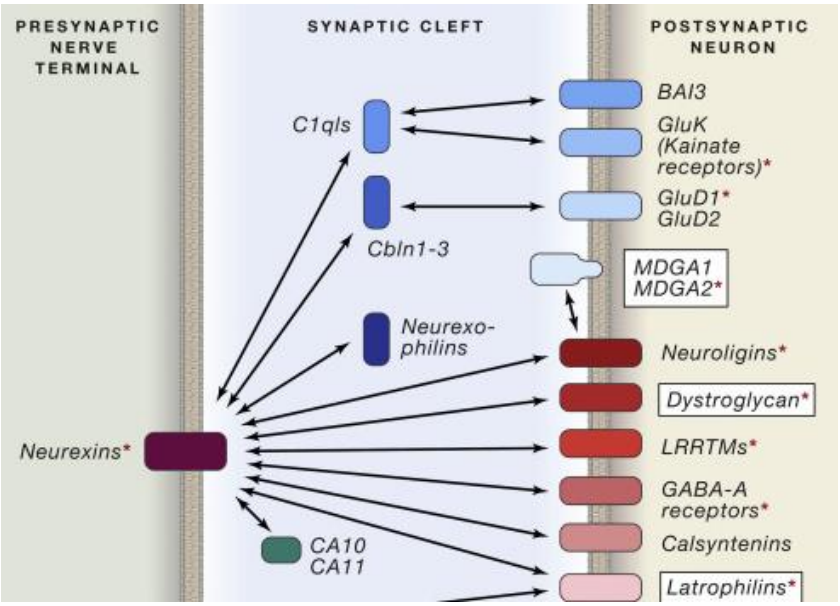

Figure 2. Schematic representation of trans-synaptic interactions between neurexins and neuroligins across the synaptic cleft. "* indicates genetic association with neuropsychiatric disorders". Figure taken from Südhof (2017).

Synapse organizers such as neurexins and neuroligins contribute to the development and plasticity of excitatory and inhibitory synapses (Connor and Siddiqui 2023; Südhof 2017). Deletion of neurexins and neuroligins has led to impaired synaptic transmission and imbalance between excitatory and inhibitory synapses (Connor and Siddiqui 2023; Südhof 2017). For example, α-neurexin KO showed a two-fold reduction in inhibitory synapses, decrease in synaptic strength and reduced action potential triggered by Ca^{2+} influx (Brockhaus et al. 2024; Bang and Owczarek, 2013). Neuroligin-2 KO displayed selective decrease in inhibitory synaptic responses (Chubykin et al. 2007). These impairments and

imbalances lead to synaptic dysfunction, which forms the basis for several neurodevelopmental disorders, particularly ASD and schizophrenia (Liu et al. 2022).

Antibodies play a crucial role in the immune response by identifying and neutralizing foreign objects such as antigens (Schrieber, 2010). Antibodies are produced by B cells and are specific to their target antigen (Schrieber, 2010). Antigens contain multiple epitopes, also known as antigenic determinants, which are the sites recognized by antibodies (Fig. 3) (Wener and Mannik, 2004). Typically, an antigen presents several epitopes on its surface (Wener and Mannik, 2004).

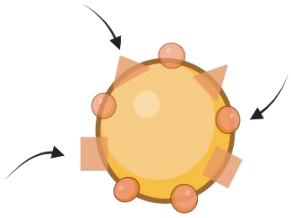

Figure 3. An antigen with epitopes on its surface (arrows showing different types of epitopes) (made from BioRender).

Antibody production is generally achieved through three primary methods, which also serve as the foundation for their commercial manufacture. Commercially produced antibodies are extensively employed in research, diagnostic applications, and therapeutic interventions (Alhazmi and Albratty, 2023).

The production of polyclonal antibodies involves immunizing animals with specific antigens at repeated intervals, thereby stimulating B cells to generate high levels of antigen-specific antibodies (Nakazawa et al. 2010). The antibodies are then extracted from

the blood (serum) and can be used in crude form or further purified (MacConmara et al. 2015). This approach mimics the natural immune response and produces a mixture of antibodies that recognize multiple epitopes (Nakazawa et al. 2010). However, polyclonal antibodies often suffer from drawbacks such as cross-reactivity, batch-to-batch variation, and reduced specificity, particularly when binding to antigens with similar sequences (MacConmara et al. 2015).

The production of monoclonal antibodies also begins with immunization but relies on the creation of hybridomas (Bayer, 2019). Hybridomas are formed by fusing B cells from an immunized animal with immortal myeloma cells, producing clones that continuously generate identical antibodies (Kothari et al. 2024). These monoclonal antibodies are homogeneous and recognize a single epitope per antigen (Karagiannis et al. 2021). Their high specificity makes them especially valuable in therapeutic applications, such as cancer treatment (Bayer, 2019; Kothari et al. 2024).

Unlike the previous two methods, recombinant antibody production is performed entirely in vitro, eliminating the need for animals (Groff et al. 2015). Antibodies are selected from gene libraries containing diverse variants, chosen for their strong binding to target antigens (Groff et al. 2015). Generation of recombinant antibodies include the use of mammalian cell lines, bacterial systems, yeast display, ribosome display, and phage display (Groff et al. 2015). Phage display, the oldest and most widely used technique, involves fusing antibody genes with phage coat protein genes so that antibodies are expressed on the phage surface (Groff et al. 2015). Mutagenesis generates vast phage display libraries containing billions of variants, which are screened through multiple rounds of selection to isolate the

strongest binders (Gera et al. 2012). These binders are then purified, amplified, and produced commercially (Gera et al. 2012).

The Institute for Protein Innovation (IPI) designs its own antigens and antibodies using recombinant methods (Faulds, n.d.). First, antigen plasmids are constructed, expressed, purified, and characterized by SDS-PAGE (Faulds, n.d.). For antibody generation, IPI utilizes yeast display (Faulds, n.d.). This process begins with the creation of a synthetic library containing a diverse repertoire of antibody genes (Mustafa et al. 2024). Antibody genes are cloned into expression vectors (plasmids) that include essential elements such as a yeast promoter, a secretion signal sequence, an antibody gene cassette, a selectable marker, and a replication origin (Mustafa et al. 2024). These vectors are introduced into yeast cells via transformation techniques such as electroporation (Gera et al. 2012).

Once transformed, yeast cells expressing antibody variants undergo screening to identify binders (Gera et al. 2012; Mustafa et al. 2024). Initial selection is performed using magnetic-activated cell sorting (MACS), in which antibodies are labeled with magnetic beads (Gera et al. 2012; Mustafa et al. 2024). This is followed by fluorescence-activated cell sorting (FACS), in which target antigens are fluorescently tagged and sorted by laser detection based on binding strength (Gera et al. 2012; Mustafa et al. 2024). FACS provides a refined second step to isolate the highest-affinity binders (Gera et al. 2012).

The best binders are then cultured, and their vector DNA is isolated and transformed into *E. coli* cells (Gera et al. 2012). The DNA is sequenced using next-generation sequencing, and the resulting sequences are used to produce recombinant monoclonal antibodies through high-throughput expression, purification, and validation (Faulds, n.d; Gera et al. 2012).

Yeast display offers several advantages over phage display (Mustafa et al. 2024). As a eukaryotic system, yeast provides machinery that supports proper folding and assembly of complex proteins such as antibodies, which is often challenging in bacterial systems used for phage display (Gera et al. 2012; Mustafa et al. 2024). Yeast can also display larger antibody fragments, whereas phage display is generally limited to smaller fragments. These features make yeast display particularly well-suited for therapeutic antibody development (Gera et al. 2012; Mustafa et al. 2024).

Antibody characterization is a method used by scientists to ensure the specificity, sensitivity, and reproducibility of antibodies used in research (Bordeaux et al. 2010). Without proper validation, antibodies may yield inaccurate or misleading results, compromising the integrity of experimental findings (Bordeaux et al. 2010). For example, a false positive may occur when an antibody binds to a molecule other than its intended target, typically due to poor specificity (Baker, 2016). Conversely, a false negative can arise when the antibody fails to recognize and bind its target antigen, reflecting insufficient sensitivity in detecting the presence of the antigen (Baker, 2016). In the research context, the consequences include wasted resources and the funding required to purchase these antibodies (Baker, 2016). In therapeutic settings, antibodies are used in cancer treatments (Bayer, 2019). Failure of these antibodies to work leads to improper treatment and ultimately patient harm.

Research objectives

1. To systemically characterize and validate commercially produced IPI antibodies using a comprehensive set of biochemical, imaging, and functional approaches.
2. To investigate the role of Nrxn1γ in maintaining shapes excitatory–inhibitory synapse balance in the adult mouse hippocampus.

Methods and materials

Immunoprecipitation

This method utilizes an antibody specific to the protein to identify the protein complexes. This method is vital in identifying a protein within a complex (Burckhardt et al. 2021). Mice were decapitated following anesthetization with isoflurane and quickly rinsed with homogenization buffer. The whole brain was homogenized in a glass-teflon homogenizer. It underwent a series of centrifugation steps and then was incubated with the detergent, Complexiolyte (CL)-47 lysis buffer containing Protease inhibitors at 4°C for one hour, followed by another centrifugation step to remove insoluble materials. The supernatant was incubated with IPI anti-Neurexin or anti-Neuroligin antibodies overnight. Following incubation, the lysates were conjugated to Sepharose G beads and then rinsed with a dilution buffer of Complexiolyte (CL)-47. The beads were boiled with Laemmli buffer before performing SDS-PAGE (Fig. 4). After SDS-PAGE, samples were blotted onto Immobilon P membranes (MilliporeSigma, IPVH85R) for western blotting. The blots were blocked in 5 % milk + Tris-buffered saline with 0.001 % Tween 20 (TBST). This step was followed by overnight incubation with the primary antibody (Anti-pan Nrx (Abn 161-I) or NLGN 1Mouse Mc Ab/Neuroligin 2 (SySy)/NLGN 3 Rabbit Poly Ab). The blot was then washed several times with TBST and subsequently incubated with the appropriate secondary antibody (anti-Rb light-chain specific/ Goat anti-mouse human ads-HRP/ Goat anti-rabbit human ads-HRP), diluted in 5% milk prepared in TBST. The secondary antibody is chosen to recognize the host species of the primary antibody specifically. Rabbit IgG was used as a negative control. After secondary incubation, the blot underwent additional TBST washes

before being imaged using the Bio-Rad imaging system to detect and analyze the presence of target protein bands (Fig. 5).

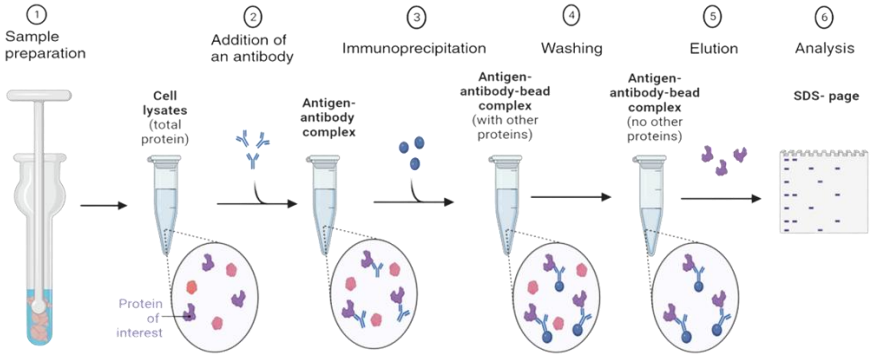

Figure 4. Schematic representation of IP. Illustrating various steps in isolating the protein of interest (made from BioRender).

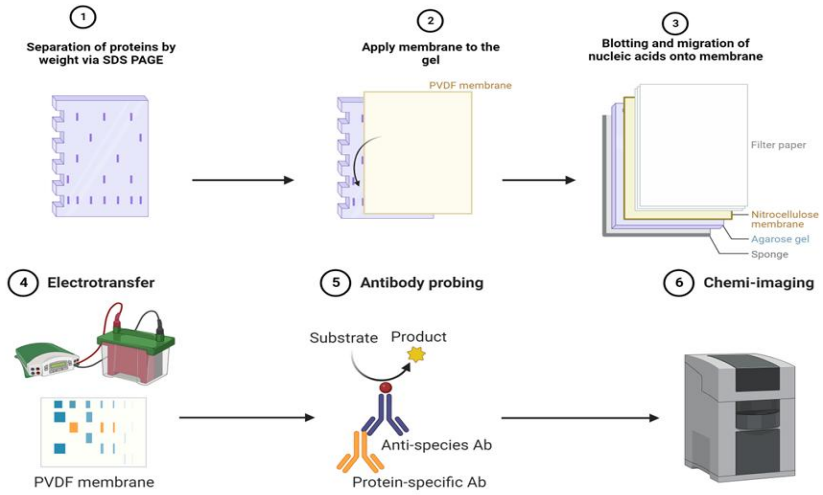

Figure 5. Schematic representation of the process of western blot. Illustrating various steps in probing the protein of interest with antibodies and detecting its presence through imaging (made from BioRender).

Antibodies

The antibodies utilized in this project were provided by Institute for Protein Innovation specifically to my advisor for testing. The antibodies were generated using yeast display. The Tab ID is included in the tables corresponding to the specific antibodies.

Neurexin1γ

As a method of demonstration for antibody validation , I have worked in this project that looked at how Neurexin1γ shapes the balance between excitatory and inhibitory synapses. Adult Nrxn1γ-deficient and wild-type littermate mice (P50–P70) were perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Brains were post-fixed overnight, cryoprotected, and coronally sectioned at 20 μm using a cryostat. Mounted hippocampal sections were permeabilized and blocked with 5% normal goat serum containing 0.3% Triton X-100, then incubated with primary antibodies against GAD65 (an inhibitory synapse marker) and VGLUT1 (an excitatory synapse marker). Following washes, sections were incubated with fluorophore-conjugated secondary antibodies. Confocal images were acquired using a 63× oil immersion objective with identical laser intensity, gain, and offset settings across genotypes to ensure comparability. These experiments and the imaging were carried out by a lab colleague as part of an ongoing project (Padmanabhan et al. 2025, unpublished). They were conducted in accordance with the standards of the Canadian Council on Animal Care (CCAC).

Statistical analysis

For Nrxn1γ project, maximum intensity projections of confocal z-stacks were generated and analyzed using MetaMorph software. The laminar boundaries of CA1 were

delineated manually, and GAD65- and VGLUT1-positive puncta were quantified using fixed thresholding and particle analysis. All image analyses were performed blinded to genotype. Results were compiled in GraphPad Prism and statistically compared using multiple t-tests with post hoc multiple-comparison corrections (Bonferroni) where appropriate.

Results

Variable detection of Neurexins with anti-Neurexin 1 antibodies

My project tested nine Nrxn1α antibodies and three Nrxn1β antibodies. The successful target detection of the antibodies was the presence of bands around 130 kDa for Nrxn1α and 130 kDa and 70 kDa for Nrxn1β. Nrxn1α 0.7 and Nrxn1α 0.21 showed no molecular bands around 130 kDa, indicating failed detection of the protein of interest (Fig. 6). Similarly, Nrxn1α 0.15 and Nrxn1α 0.2 show no distinct bands, suggesting their failure in detecting the Nrxn1α isoform (Fig. 7). Nrxn1α 0.43, Nrxn1α 0.40 and Nrxn1α 0.45 displayed no distinct bands (Fig. 8). However, Nrxn1α 0.40 displayed a single distinct band around 130 kDa in the third trial (Fig. 9), after two false negatives (Fig. 8 and Fig.10). This suggests that Nrxn1α 0.40 required higher sample load or more optimized conditions to achieve reliable detection, rather than representing a true negative. In contrast, Nrxn1α 0.9 showed no distinct band indicating its failure to detect the protein of interest (Fig. 10).

Nrxn1β 0.29 and Nrxn1β 0.35 displayed successful target protein detections with bands around 130 kDa and 70 kDa (Fig. 10). In the second trial, Nrxn1β 0.29 and Nrxn1β 0.35 detected the target protein, but the bands are hard to visualize (Fig. 11). On the contrary, Nrxn1β 0.13 showed no band on both trials (Fig. 8 and Fig.11) (Table 1).

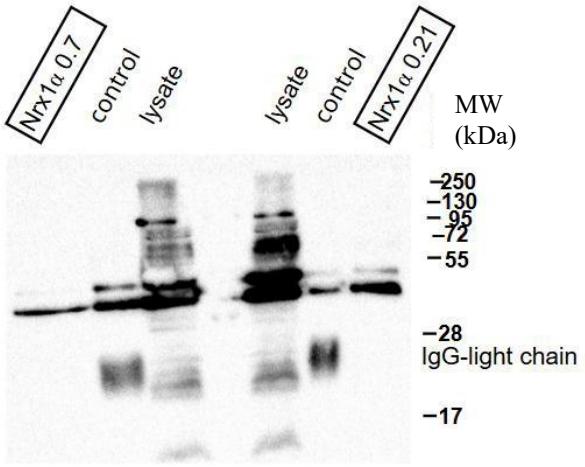

Figure 6. Western blot analysis of Nrxn1α 0.7 and Nrxn1α 0.21 antibodies. No distinct bands at 130 kDa were detected, indicating failed immunoprecipitation of Nrxn1α. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

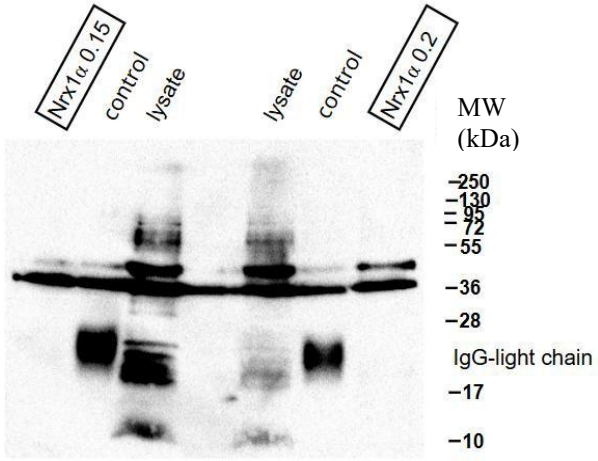

Figure 7. Western blot analysis of Nrxn1α 0.15 and Nrxn1α 0.2 antibodies. No distinct bands at 130 kDa were detected, indicating failed immunoprecipitation of Nrxn1α. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

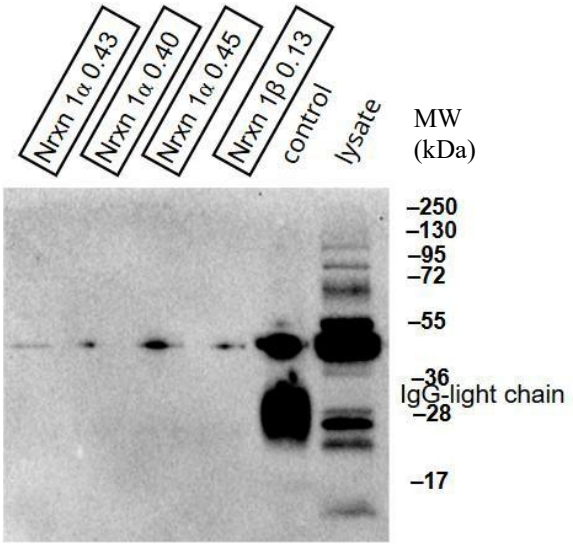

Figure 8. Western blot analysis of multiple Nrxn1 antibodies. No distinct bands at 130 kDa or 70 kDa were detected for Nrxn1α 0.43, Nrxn1α 0.40, Nrxn1α 0.45, and Nrxn1β 0.13 antibodies. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

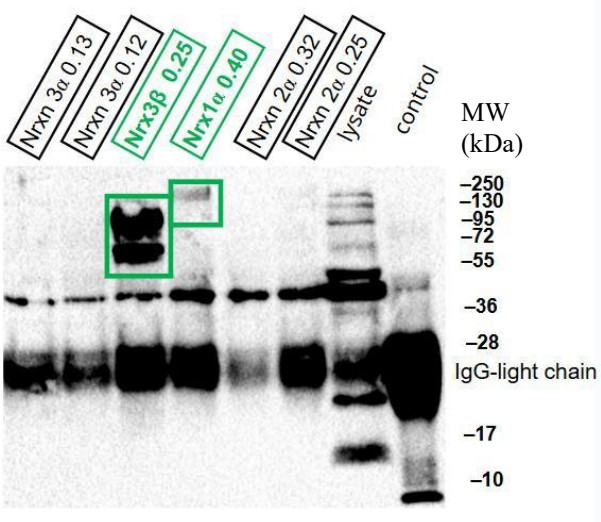

Figure 9. Western blot analysis of multiple neurexin antibodies. Nrxn3β displayed prominent bands at ~130 kDa and ~70 kDa (green box). Nrxn2α 0.25 and Nrxn2α 0.32 showed no bands in this trial. Nrxn3α 0.13, Nrxn3α 0.12, and Nrxn1α 0.40 showed no detectable bands. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

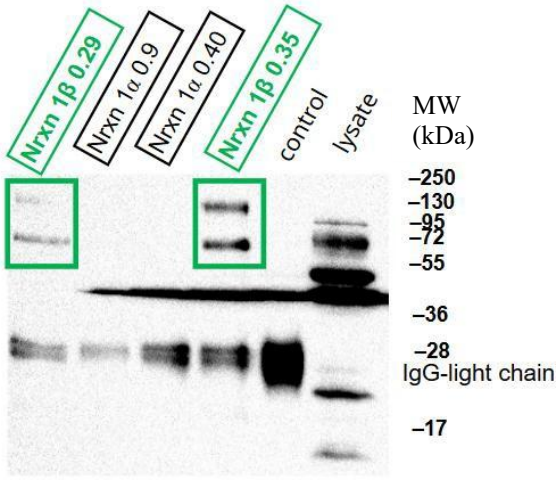

Figure 10. Western blot analysis of Nrxn1α and Nrxn1β antibodies. Nrxn1β 0.29 and Nrxn1β 0.35 showed distinct bands at ~130 kDa and ~70 kDa (green boxes), indicating successful detection. Nrxn1α 0.9 and Nrxn1α 0.40 showed no detectable bands. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

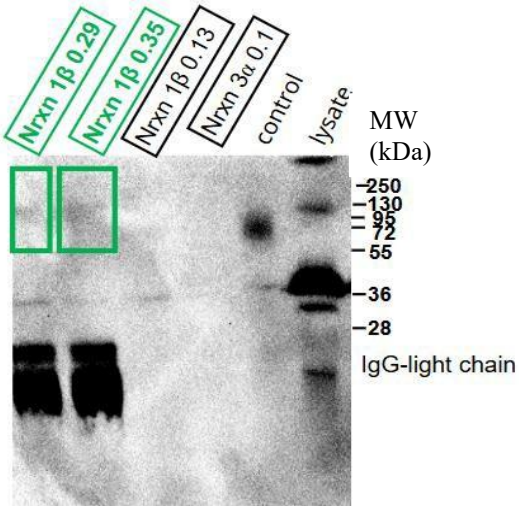

Figure 11. Western blot analysis of Nrxn1β and Nrxn3α antibodies. Nrxn1β 0.29 and Nrxn1β 0.35 showed bands at ~130 kDa (green boxes), though weaker than trial 1. Nrxn1β 0.13 and Nrxn3α 0.1 showed no detectable bands. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

Table 1. Effectiveness of Neurexin 1 antibodies tested across various trials. Antibody performance was assessed across three experimental trials (n, 2n and 3n) with successful detection indicated by prominent Neurexin-1 bands at approximately 70 kDa and 130 kDa. "X" denotes trials that were not performed. TAB ID indicates the unique serial number. (+) indicates successful detection. (-) indicates unsuccessful detection.

TAB ID	Antibodies tested	Target protein detected		
		n	2n	3n
N/A	Nrxn 1α 0.2	-	X	X
N/A	Nrxn 1α 0.7	-	X	X
N/A	Nrxn 1α 0.9	-	X	X
N/A	Nrxn 1α 0.15	-	X	X
TAB0014761-013	Nrxn 1α 0.21	-	X	X
TAB0013427-013	Nrxn 1α 0.40	-	-	+
TAB0013430-013	Nrxn 1α 0.43	-	X	X
TAB0013432-013	Nrxn 1α 0.45	-	X	X
TAB0012053-013	Nrxn 1β 0.13	-	-	X
TAB0013511-013	Nrxn 1β 0.29	+	+	X
TAB0013517-013	Nrxn 1β 0.35	+	+	X

Immunodetection of Neurexin 2α antibodies

Nrxn 2α 0.2 and Nrxn2α 0.13 failed to display bands depicting the molecular protein of interest (Fig. 12). Nrxn2α 0.25 and Nrxn 2α 0.32 showed promising results in the first trial, with clear bands across 130 kDa and 70 kDa (Fig. 12). However, there is a notable absence of these bands on the second trial (Fig. 9). This could be due to the samples being stored for two weeks at 4 degrees prior use. This could have led to degradation of the sample prior to the SDS-PAGE (Table 2).

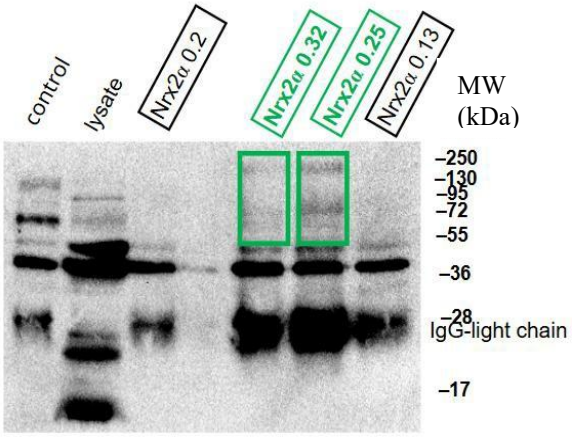

Figure 12. Western blot analysis of Nrxn2α antibodies. Nrxn2α 0.32 and Nrxn2α 0.25 showed prominent bands around 130 kDa and 70 kDa (green boxes), indicating successful detection. Nrxn2α 0.2 and Nrxn2α 0.13 showed no detectable bands. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

Table 2. Effectiveness of Neurexin 2 antibodies tested across various trials. Antibody performance was assessed across two experimental trials (n and 2n), with successful detection indicated by prominent Neurexin-2 bands at approximately 70 kDa and 130 kDa. "X" denotes trials that were not performed. TAB ID indicates the unique serial number. (+) indicates successful detection. (-) indicates unsuccessful detection.

TAB ID	Antibodies tested	Target protein detected	
		n	2n
TAB0013303-013	Nrxn 2α 0.2	-	X
TAB0013314-013	Nrxn 2α 0.13	-	X
TAB0013326-013	Nrxn 2α 0.25	+	-
TAB0013333-013	Nrxn 2α 0.32	+	-

Differential detection of neurexin 3 through anti-Neurexin 3 antibodies

Nrxn 3α 0.1, Nrxn 3α 0.4 and Nrxn 3α 0.67 all failed to show consistent bands around 130 kDa (Fig. 11, Fig. 13 and Fig. 14). Similarly, Nrxn 3α 0.12 and Nrxn 3α 0.13 failed to show bands around 130 kDa for both trials (Fig. 9 and Fig. 14). These results indicate the inability of the antibodies to detect Nrxn 3α isoforms.

Nrxn 3β 0.31 showed no visible bands, indicating failure of protein detection (Fig. 13). However, Nrxn3β 0.21 and Nrxn3β 0.25 showed strong and prominent bands at both 130 kDa and 70 kDa, suggesting that the beta isoforms of Nrxn3 were highly effective and may serve as a preferred antibody for detecting Neurexin 3β (Fig. 9, Fig. 13 and Fig. 14) (Table 3).

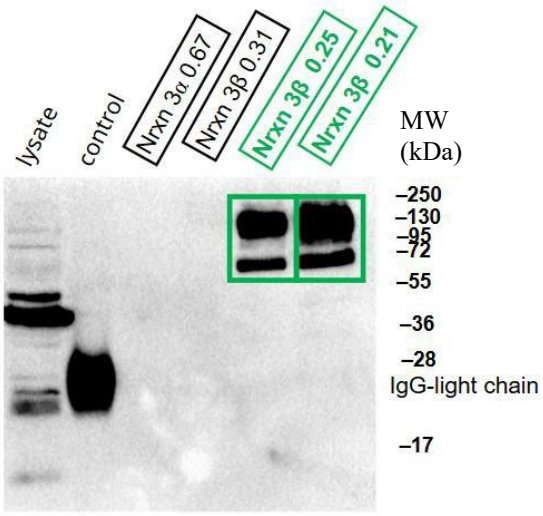

Figure 13. Western blot analysis of Nrxn3α and Nrxn3β antibodies. Nrxn3β 0.25 and Nrxn3β 0.21 showed strong bands at ~130 kDa and ~70 kDa (green box), indicating successful detection. Nrxn3α 0.67 and Nrxn3β 0.31 showed no detectable bands. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

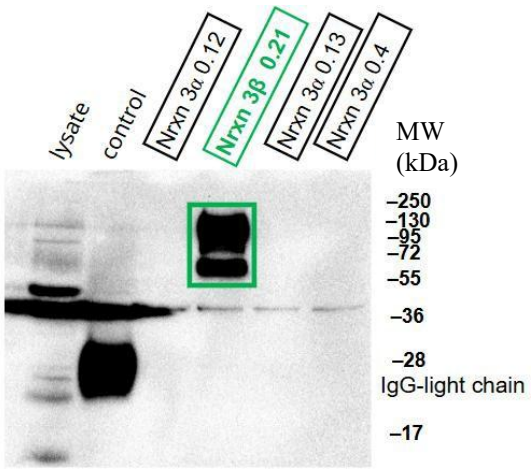

Figure 14. Western blot analysis of Nrxn3 antibodies. Nrxn3β 0.21 showed strong bands at ~130 kDa and ~70 kDa (green box), indicating successful detection. Nrxn3α 0.12, Nrxn3α 0.13 and Nrxn3α 0.4 showed no detectable bands. Anti-Pan Nrx (Abn161-I) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control

Table 3. Effectiveness of Neurexin 3 antibodies tested across various trials Antibody performance was assessed across two experimental trials (n and 2n), with successful detection indicated by prominent Neurexin-3 bands at approximately 70 kDa and 130 kDa. "X" denotes trials that were not performed. TAB ID indicates the unique serial number. (+) indicates successful detection. (-) indicates unsuccessful detection.

TAB ID	Antibodies tested	Target protein detected	
		n	2n
TAB0011968-013	Nrxn 3α 0.1	-	X
TAB0011971-013	Nrxn 3α 0.4	-	X
TAB0011979-013	Nrxn 3α 0.12	-	-
TAB0011980-013	Nrxn 3α 0.13	-	-
TAB0014903-013	Nrxn 3α 0.67	-	X
TAB0014854-013	Nrxn 3β 0.21	+	+
N/A	Nrxn 3β 0.25	+	+
TAB0014864-013	Nrxn 3β 0.31	-	X

Variable detection of Neuroligin 1 utilizing anti- Neuroligin 1 antibodies

Nlgn 1.7 showed a prominent single band around 100 KDa for both trials, which aligns with the molecular weight of the neuroligin antibodies (Fig. 15 and Fig. 16) Similarly Ngln 1.11 showed a consisted band around 100 kDa for both trials (Fig. 15 and Fig. 16). Nlgn 1.1 shows a distinct band around 100 kDa for both trials, indicating successful detection of the target protein (Fig. 16 and Fig. 17). Nlgn 1.5 and 1.36 failed to show visible bands for both trials (Fig. 15, Fig.16 and Fig. 18). Nlgn 1.12, Nlgn 1.15 and Nlgn 1.31 do not show visible bands for both trials, indicating failure to detect the Nlgn protein (Fig. 17 and Fig. 18). Lastly, Nlgn 1.10 displayed a distinct band around 100 kDa, indicating successful detection of the protein of interest for both trials (Fig. 16 and Fig. 19) (Table 4).

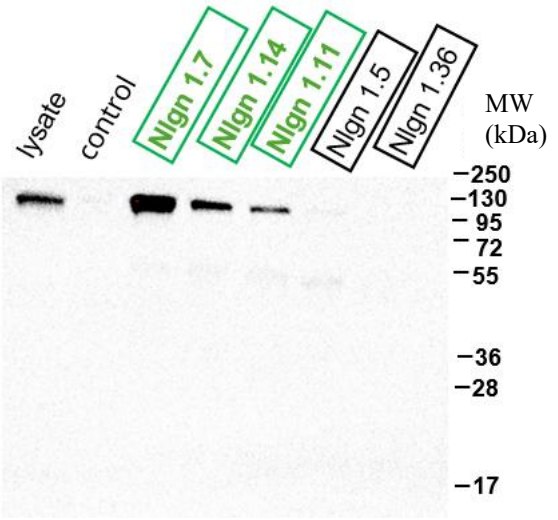

Figure 15. Western blot analysis of Nlgn1 antibodies. Nlgn 1.7, Nlgn 1.14, and Nlgn 1.11 showed distinct bands at ~100 kDa (green boxes), indicating successful detection. Nlgn 1.5 and Nlgn 1.36 showed no detectable bands. NLGN1 Mouse McAb primary; goat anti-mouse HRP secondary; Rb IgG as negative control

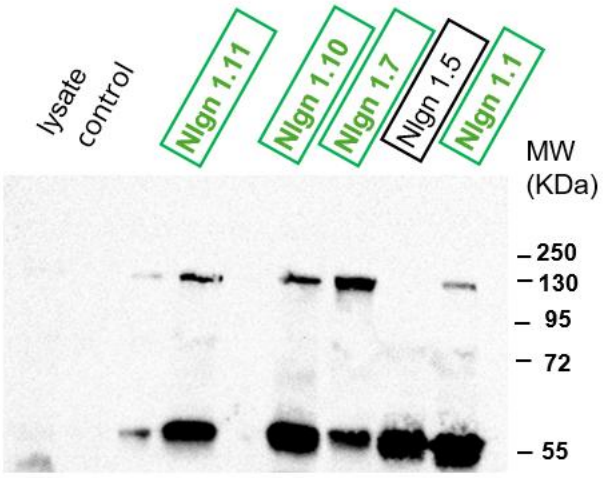

Figure 16. Western blot analysis of Nlgn1 antibodies. Nlgn 1.11, Nlgn 1.10, Nlgn 1.7, and Nlgn 1.1 showed bands at ~100 kDa (green boxes). Nlgn 1.5 showed no detectable band. NLGN1 Mouse McAb primary; goat anti-mouse HRP secondary; Rb IgG as negative control.

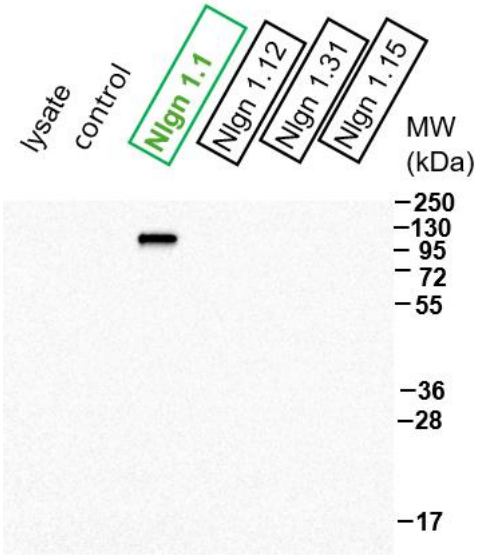

Figure 17. Western blot analysis of Nlgn1 antibodies. Nlgn 1.1 showed a single distinct band at ~100 kDa (green box), indicating successful detection. Nlgn 1.12, Nlgn 1.31, and Nlgn 1.15 showed no detectable bands. NLGN1 Mouse McAb primary; goat anti-mouse HRP secondary; Rb IgG as negative control.

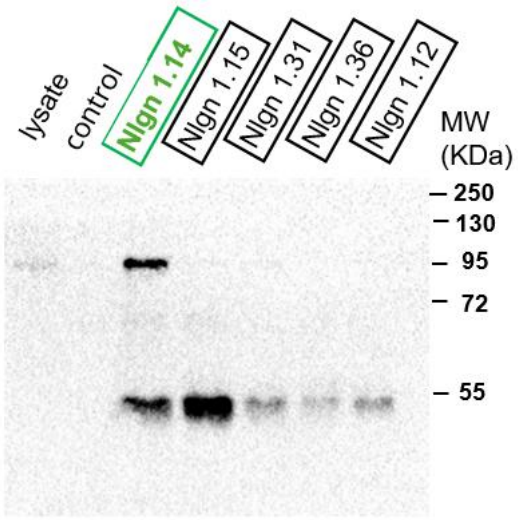

Figure 18. Western blot analysis of Nlgn1 antibodies. Nlgn 1.14 showed a distinct band at ~100 kDa (green box). Nlgn 1.15, Nlgn 1.31, Nlgn 1.36, and Nlgn 1.12 showed no detectable bands at 100 kDa. NLGN1 Mouse McAb primary; goat anti-mouse HRP secondary; Rb IgG as negative control.

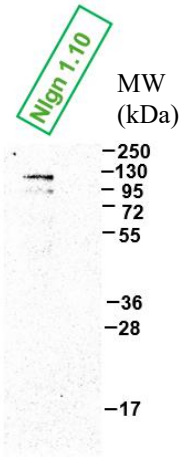

Figure 19. Western blot analysis of Nlgn 1.10 antibody. A distinct band at ~100 kDa was detected, indicating successful immunoprecipitation of Nlgn1. NLGN1 Mouse McAb primary; goat anti-mouse HRP secondary; Rb IgG as negative control

Table 4. Effectiveness of different variants of Neuroligin 1 antibodies tested. Antibody performance was assessed across two experimental trials (n and 2n), with successful detection indicated by prominent Neuroligin-1 band at approximately 100 kDa. TAB ID indicates the unique serial number. (+) indicates successful detection. (-) indicates unsuccessful detection.

TAB ID	Antibodies tested	Target protein detected	
		n	2n
TAB0013463-013	NLGN 1.1	+	+
TAB0013467-013	NLGN 1.5	-	-
TAB0013469-013	NLGN 1.7	+	+
TAB0013472-013	NLGN 1.10	+	+
TAB0013473-013	NLGN 1.11	+	+
TAB0011945-013	NLGN 1.12	-	-
TAB0013476-013	NLGN 1.14	+	+
TAB0013477-013	NLGN 1.15	-	-
TAB0014673-013	NLGN 1.31	-	-
N/A	NLGN 1.36	-	-

Consistent detection of target protein with Neuroligin 2 antibodies

Among the three Nlgn 2 antibodies tested, Nlgn 2.2, Nlgn 2.4 and Nlgn 2.1, all displayed a strong band around the molecular weight of the 100 kDa in both n and 2n sample, indicating the reliable detection of Nlgn 2 (Fig. 20 and Fig 21) (Table 5).

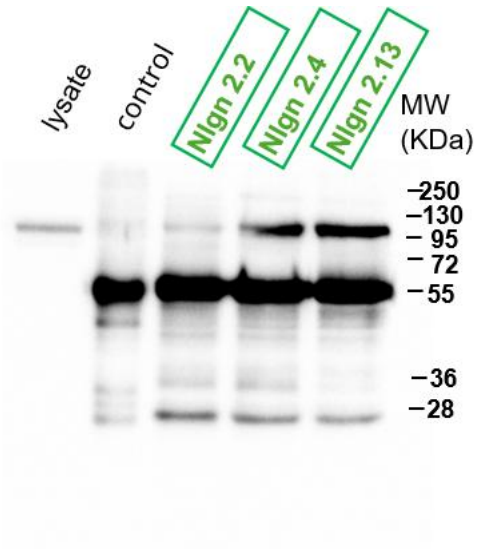

Figure 20. Western blot analysis of Nlgn2 antibodies. Nlgn 2.2, Nlgn 2.4, and Nlgn 2.13 all showed prominent bands at ~100 kDa (green boxes), confirming detection of Nlgn2. Neuroligin 2 (SySy) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

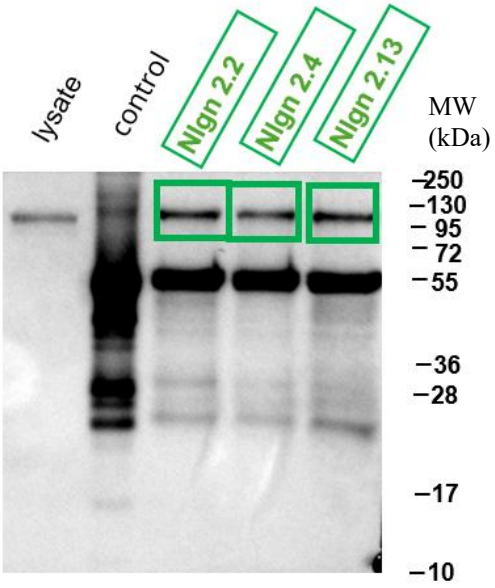

Figure 21. Western blot analysis of Nlgn2 antibodies in second trial. Nlgn 2.2, Nlgn 2.4, and Nlgn 2.13 all showed prominent bands at ~100 kDa (green boxes), confirming consistent and reliable detection of Nlgn2. Neuroligin 2 (SySy) primary; anti-Rb light chain-specific secondary; Rb IgG as negative control.

Table 5. Effectiveness of Neuroligin 2 antibodies tested across various trials. Antibody performance was assessed across two experimental trials (n and 2n), with successful detection indicated by prominent Neuroligin-2 band at approximately 100 kDa. TAB ID indicates the unique serial number. (+) indicates successful detection. (-) indicates unsuccessful detection.

TAB ID	Antibodies tested	Target protein detected	
		n	2n
TAB0011863-013	NLGN 2.2	+	+
TAB0011256-013	NLGN 2.4	+	+
TAB0011874-013	NLGN 2.13	+	+

Successful validation of neuroligin 3 antibodies

Nlgn 3.3, Nlgn 3.5, Nlgn 3.7, Nlgn 3.18 and Nlgn 3.19 were all the neuroligin 3 antibodies tested and they all show prominent band congruent with the molecular weight of the neuroligin proteins around 100 kDa for both trials (Fig. 22 and Fig. 23) (Table 6). This indicates that any of the mentioned antibodies can serve to reliably detect Nlgn 3.

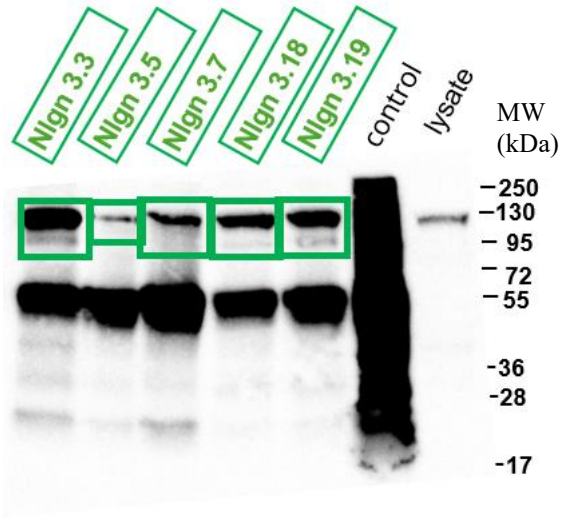

Figure 22. Western blot analysis of Nlgn3 antibodies. Nlgn 3.3, Nlgn 3.5, Nlgn 3.4, Nlgn 3.18, and Nlgn 3.7 all displayed prominent bands at ~100 kDa (green box), indicating successful detection of Nlgn3. NLGN3 Rabbit PolyAb primary; goat anti-rabbit HRP secondary; Rb IgG as negative control.

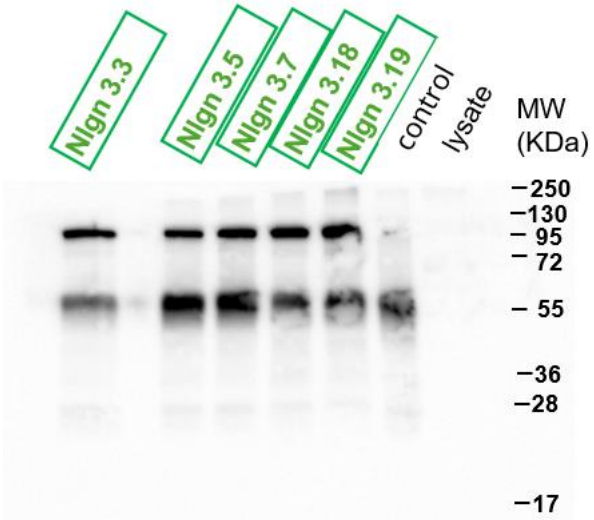

Figure 23. Western blot analysis of Nlgn3 antibodies in second trial. Nlgn 3.3, Nlgn 3.5, Nlgn 3.7, Nlgn 3.18, and Nlgn 3.19 all showed consistent bands at ~100 kDa confirming reproducible detection of Nlgn3 across trials. NLGN3 Rabbit PolyAb primary; goat anti-rabbit HRP secondary; Rb IgG as negative control.

Table 6. Effectiveness of Neuroligin 3 antibodies tested. Antibody performance was assessed across two experimental trials (n and 2n), with successful detection indicated by prominent Neuroligin-3 band at approximately 100 kDa. TAB ID indicates the unique serial number. (+) indicates successful detection. (-) indicates unsuccessful detection.

TAB ID	Antibodies tested	Target protein detected	
		n	2n
TAB0011271-013	NLGN 3.3	+	+
TAB0011273-013	NLGN 3.5	+	+
TAB0011275-013	NLGN 3.7	+	+
TAB0011949-013	NLGN 3.18	+	+
TAB0011950-013	NLGN 3.19	+	+

Neurexin 1γ plays a critical role in maintaining an excitation/inhibition balance (E/I)

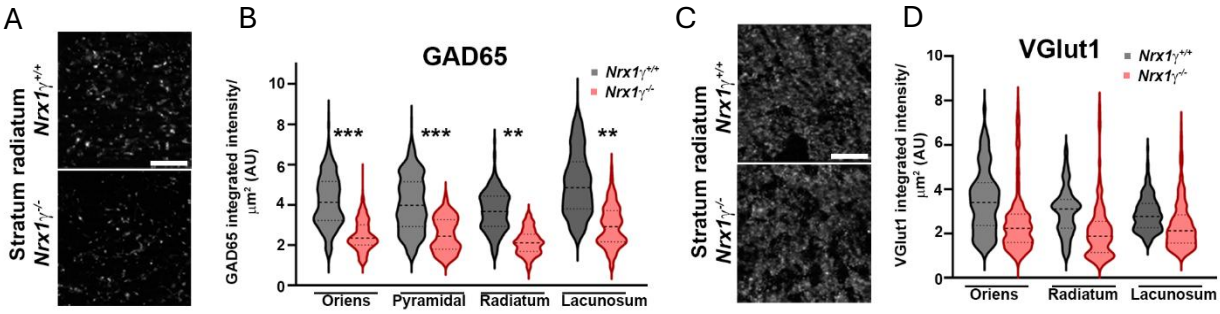

Figure 24. Reduced inhibitory but not excitatory synaptic input in Nrx1γ$^{-/-}$ mice.
A. Representative confocal images from the pyramidal layer of hippocampal CA1 showing GAD65 punctate signal significantly reduced in knockout (Nrx1γ$^{-/-}$, bottom) as compared to wild-type (Nrx1γ$^{+/+}$, top) mice.
B. Quantification of GAD65 puncta per intensity area in Oriens, Radiatum, Pyramidal and Lacunosum layers show significant difference between wild-type (Nrx1γ$^{+/+}$, grey) and knockout (Nrx1γ$^{-/-}$, orange); 375-443 fields/regions, n=3-4 mice/genotype.
C. Vglut1 puncta in the CA1 remain unchanged between wild-type (Nrx1γ$^{+/+}$, top) and knockout (Nrx1γ$^{-/-}$, bottom) mice.
D. Quantification of Vglut1 puncta per intensity area in Oriens, Radiatum, and Lacunosum layers show no significant difference between wild-type (Nrx1γ$^{+/+}$, grey) and

knockout (Nrx1γ$^{-/-}$,orange); 321-410 field/regions, n=4 mice/genotype. Statistical analysis was performed using multiple t-tests with Bonferroni correction (**p<0.01, ***p<0.001)

A quantitative analysis of synaptic immunofluorescence puncta was performed in the dorsal CA1 to analyze the excitation/inhibition balance in Nrx1γ$^{-/-}$ mice. As illustrated in Fig. 24, the ablation of Nrx1γ resulted in reduced density of the GAD65 positive puncta across all the CA1 strata, indicating selective impairment in inhibitory synapses. In contrast, the VGLUT1 positive puncta remain relatively unchanged in respect to the controls, suggesting no impairment in the excitatory synapses. These results indicate that Nrx1γ possess important role in inhibitory synapse growth and maintenance.

Discussion

This project tested different variants of neurexins and neuroligin antibodies by the methods of immunoprecipitation, followed by western blot.

Presence of smear for neurexins and not neuroligins

For a western blot, an indication of the antibodies binding to your proteins of interest is distinct bands concurrent to the molecular weight of the protein of interest (Yang and Mahmood 2012). An indication of a smear either points towards the protein of interest being degraded or sample overload (Butler et al. 2019). In case of neurexins, the smear is due to the heparin sulphate chains (Zhang et al. 2018). On the contrary, neuroligin do not possess such chains and appear as a distinct band (Craig and Kang 2007).

Why is validation of neurexin and neuroligin antibodies important?

Historically, the absence of well-validated antibodies against neurexins and neuroligins has represented a significant barrier for progress in synaptic biology (Boxer and Aoto 2022). For example, reports suggesting that neurexin expressed outside glial and neuronal cells could not be validated due to the lack of reliable antibodies (Südhof 2017). Another example is the difficulty in localizing endogenous neurexin due to the lack of validated antibodies that can distinguish between the various isoforms (Klatt et al. 2021). Ducrot et al. (2025) created a knock in model of endogenous tagged neuroligin to study its function. This process was created due to a lack of reliable antibodies against neuroligin. In addition to this, both neurexins and neuroligins contain alternative splicing sites which lead to multiple splice variants of the proteins, which identified through antibody validation can serve as biomarkers for studying synaptic pathology in diseases such as Alzheimer's disease (AD) (Camporesi 2021).

Applications of validated neurexin and neuroligin antibodies

Commercially produced antibodies are often at risk of not recognizing the target proteins. According to Groff et al. (2015), only 49% of the commercially produced antibodies tested worked. Validating antibodies ensures that the antibodies used in the experiments produce results that are congruent with the experimental setting rather than due to the failure of target detection (Taussig et al. 2018). This project took a preliminary step towards ensuring that the research is not hindered due to the absence of reliable antibodies. The validation of antibodies in this project can be used to create antibody databases, which can be accessed by the public (Taussig et al. 2018). For example, Structural antibody database, known as SAbDab is an online resource that contains specific

and general information about the antibodies (Dunbar et al.,2013). Similar to my project, validated neurexin and neuroligin antibodies can be used in western blot to quantify protein expression. This can be helpful to measure protein expression in knock out model, knock in or a diseased model (Padmanabhan et al. 2025, unpublished). Antibodies used in techniques such as immunohistochemistry and immunocytochemistry can be used to visualize and localize neurexins and neuroligins. This can be helpful in studying synapse development and maturation (Harkin et al. 2016). Antibodies against neuroligin and neurexin can also be used to block the binding of synaptic toxins, such as Aβ oligomers to the hippocampal neurons and prevent synapse loss (Brito-Moreira et al. 2017). Furthermore, antibodies can also help detect the presence of specific isoforms and their functions, for example, using antibodies to detect the presence of Neurexin 1γ and their expression across the brain (Padmanabhan et al. 2025, unpublished). Finally, antibodies are important for studying protein-protein interactions through co-immunoprecipitation. For example, studying the presence of neuroligins in immunoprecipitates that were specifically tagged for neurexin isoforms (Arias-Aragón et al. 2025).

Limitations of antibody validation

Epitope masking and post-translation modifications could result in poor antibody performance

Neurexin and neuroligin possess alternative splicing patterns, leading to formation of multiple isoforms. Neurexins, in particular possess extensive splicing pattern, potentially leading to over 4000 different variants (Fuccillo & Pak, 2021). It is highly probable that from the numerous antibodies tested, some variants could not recognize the protein. This could be partly due to the mechanisms of antibody-antigen binding, as the antibody

recognizes specific epitopes present on the protein. The IPI team modeled the antibodies targeting the epitopes that protrude into the extracellular part of the membrane (Halber, 2025). If the epitope lies near or within a splice-variable region, some isoforms will not contain the sequence that contains epitopes. Neurexins also possess epitopes intracellularly, but it's mainly the extracellular ones that are targeted by antibodies (Fairless et al., 2008). Similarly, the extracellular domain of the neuroligin is the one recognized by the antibodies (Craig and Kang 2007). The extracellular N terminal is heavily glycosylated which could lead to the epitope being masked, leading to the failure of the antibody (Lin et al. 2023). This glycosylation is vital for transport and responsible for the locular weight of the neuroligin (Bemben et al., 2015). The glycosylation is also isoform specific, additionally could be the reason for some isoforms performing better than others (Bemben et al., 2015).

Antibody affinity and low abundance pose a hindrance in antibody performance

Antibody affinity is defined as the binding strength of the antibody to the protein of interest (Wark & Hudson, 2006). In the process of developing antibodies, the antibodies undergo the step of affinity maturation, where higher affinity can be achieved by introducing diversity in the sequence of the antibody (Groff et al., 2015). Diversity leads to enhancing established interactions or promoting new interactions between antigen and complementarity-determining regions (CDRs) . This is usually done by steps such as DNA shuffling and error prone PCR, but this introduces a tradeoff where mutations are introduced, leading to decreased antibody stability (Groff et al., 2015). Similarly, achieving antibody specificity while established affinity is rare, even after affinity maturation (Rabia et al. 2018). According to Rabia et al. (2018), one of the antibodies showed weakened specificity even with high affinity. Increased antibody affinity could also lead to decreased

solubility (Rabia et al. 2018) . All these tradeoffs mentioned could provide explanations of why some of the antibodies highlighted in table 1-7 did not perform well.

The abundance of the protein in the lysates could be another factor to explain some of the antibodies not being able to detect the protein of interest. Neurexins are generally described as low-abundance synaptic proteins which could contribute to protein not being pulled down successfully by some of the antibodies (Vicidomini et al. 2024). Combining this with potential problems, such as decrease in antibody affinity could further explain the poor performance for some of these antibodies.

False negatives and false positives in antibody validation

Antibody-based detection of neurexins and neuroligins is vulnerable to both false positives and false negatives due to the complex biology of these synaptic adhesion molecules. False negative usually arise when the antibody concentration is low or due to the poor sensitivity of the antibody to the antigen (Reverberi and Reverberi 2007). This seems to be the case for Neurexin 1α 0.40, as it yielded false negatives in the first two trials (Fig. 8 and Fig. 10). Particularly for this, the antibody concentration might have played a role. For the third trial, the antibody concentration was increased, leading to a successful detection (Fig. 9). False positives arise mostly due to poor specificity of the antibody where it detects proteins that are not the intended target (Reverberi and Reverberi 2007). This usually arises when the antibody intended for a specific isoform detects another isoform or the antibody detects an unrelated protein that shares similar sequence with the protein of interest (Reverberi and Reverberi 2007). Usually, antibodies are further tested in knockout models to ensure that the results are not due to false positives or negatives.

Impact of Nrxn1γ ablation in E/I balance.

Previous studies have established that neurexins are critical regulators of synaptic transmission, with α- and β-neurexins playing vital roles in both excitatory and inhibitory synapses. In particular, deletion of all α-neurexins resulted in a two-fold decrease in inhibitory synapses, whereas excitatory synapses were not affected (Bang and Owczarek, 2013). By contrast, ablation of Nrxn1α led to a decrease in basal excitatory synaptic strength, while inhibitory synapses remained comparable to wild type (Etherton et al. 2009). Knockout of β-neurexins caused a reduction in excitatory synaptic strength and transmission (Klatt et al. 2021; Brockhaus et al. 2024; Anderson et al. 2015). Additionally, mutations affecting β-Nrxn1 isoforms, alone or in conjunction with impaired α-Nrxn1 function led to disruption in E/I (Excitation/inhibition) balance. However, the specific contribution of the γ isoform has remained poorly defined. By demonstrating that Nrxn1γ ablation disrupts inhibitory synapse growth, these findings provide direct evidence that this isoform is not redundant but instead serves a specialized role in maintaining inhibitory connectivity. This aligns with emerging literature suggesting that neurexin isoforms act in a synapse-type- and brain-region-specific manner, underscoring the importance of considering isoform diversity when investigating mechanisms of excitatory–inhibitory balance (Boxer and Aoto, 2022).

Taken together, my project bridges a methodological gap by validating antibody tools for synaptic protein analysis and a mechanistic gap by revealing the unique role of Nrxn1γ in inhibitory synapse stability, thereby contributing to a more nuanced model of neurexin-mediated synaptic regulation.

Future Directions

A logical next step in advancing this project will be to expand antibody validation for neurexin and neuroligin beyond western blot. While the current results establish a strong foundation, future work should incorporate validation strategies that can independently confirm antibody specificity and performance. Mass spectrometry-based analyses, such as immunoprecipitation followed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), would allow direct identification of bound protein targets and help distinguish between closely related neurexin and neuroligin isoforms. In addition, extending validation into cellular and neuronal systems through immunocytochemistry would provide critical insight into antibody behaviour in a biologically relevant context, including subcellular localization, signal fidelity, and potential off-target labeling. Integrating these complementary approaches would create a more comprehensive validation pipeline and ultimately strengthen the reliability of antibodies used to investigate neurexin–neuroligin function in synaptic biology.

Literature Cited

Alhazmi, H. A., and Albratty, M. 2023. Analytical techniques for the characterization and quantification of monoclonal antibodies. Pharmaceuticals. **16**(2): 291. https://doi.org/10.3390/ph16020291

Anderson, G. R., Aoto, J., Tabuchi, K., Földy, C., Covy, J., Yee, A. X., Wu, D., Lee, S., Chen, L., Malenka, R. C., and Südhof, T. C. 2015. B-Neurexins control neural circuits by regulating synaptic endocannabinoid signaling. Cell. **162**(3): 593–606. https://doi.org/10.1016/j.cell.2015.06.056

Arias-Aragón, F., Robles-Lanuza, E., Sánchez-Gómez, Á., Martinez-Mir, A., and Scholl, F. G. 2025. Analysis of neurexin-neuroligin complexes supports an isoform-specific role for beta-neurexin-1 dysfunction in a mouse model of autism. Molecular Brain. **18**(1): 20. https://doi.org/10.1186/s13041-025-01183-0

Baker, M. 2016. Many researchers fail to validate antibodies for their experiments. Nature. https://doi.org/10.1038/nature.2016.20192

Bang, M. L., and Owczarek, S. 2013. A matter of balance: Role of neurexin and neuroligin at the synapse. Neurochemical Research. **38**(6): 1174–1189. https://doi.org/10.1007/s11064-013-1029-9

Basha, M.A. 2024. Mechanisms of laminar organization of hippocampal excitatory synapses. M.Sc. thesis, Department of Physiology and Pathophysiology, The University of Manitoba, Winnipeg, M.B.

Bayer, V. 2019. An overview of monoclonal antibodies. Seminars in Oncology Nursing. **35**(5), 150927. https://doi.org/10.1016/j.soncn.2019.08.006

Bemben, M. A., Shipman, S. L., Nicoll, R. A., and Roche, K. W. 2015. The cellular and molecular landscape of neuroligins. Trends in Neurosciences. **38**(8): 496–505. https://doi.org/10.1016/j.tins.2015.06.004

Bittmann, S., Luchter, E., and Alieva, E.M. 2025. The Role of SAM`s as Key Player in Synapse Formation and Dysfunction: Make a Synapse, Shape a Synapse. Am J Biomed Sci & Res. **29**(3). http://10.0.133.249/AJBSR.2025.29.003800

Bordeaux, J., Welsh, A. W., Agarwal, S., Killiam, E., Baquero, M. T., Hanna, J. A., Anagnostou, V. K., and Rimm, D. L. 2010. Antibody validation. BioTechniques. **48**(3): 197–209. https://doi.org/10.2144/000113382

Bottos, A., Rissone, A., Bussolino, F., and Arese, M. 2011. Neurexins and neuroligins: synapses look out of the nervous system. Cellular and Molecular Life Sciences. **68**(16): 2655–2666. https://doi.org/10.1007/s00018-011-0664-z

Boxer, E. E., and Aoto, J. 2022. Neurexins and their ligands at inhibitory synapses. Front. Synaptic Neurosci. **14**:1087238. https://doi.org/10.3389/fnsyn.2022.1087238

Brito-Moreira, J., Lourenco, M. V., Oliveira, M. M., Ribeiro, F. C., Ledo, J. H., Diniz, L. P., Vital, J. F., Magdesian, M. H., Melo, H. M., Barros-Aragão, F., De Souza, J. M., Alves-Leon, S. V., Gomes, F. C., Clarke, J. R., Figueiredo, C. P., De Felice, F. G., and Ferreira, S. T. 2017. Interaction of amyloid-β (Aβ) oligomers with neurexin 2α and neuroligin 1 mediates synapse damage and memory loss in mice. Journal of Biological Chemistry. **292**(18): 7327–7337. https://doi.org/10.1074/jbc.m116.761189

Brockhaus, J., Kahl, I., Ahmad, M., Repetto, D., Reissner, C., and Missler, M. 2024. Conditional knockout of neurexins alters the contribution of calcium channel subtypes to presynaptic CA2+ influx. Cells. **13**(11): 981. https://doi.org/10.3390/cells13110981

Burckhardt, C. J., Minna, J. D., and Danuser, G. 2021. Co-immunoprecipitation and semi-quantitative immunoblotting for the analysis of protein-protein interactions. STAR Protocols. **2**(3): 100644. https://doi.org/10.1016/j.xpro.2021.100644

Butler, T. A. J., Paul, J. W., Chan, E., Smith, R., and Tolosa, J. M. 2019. Misleading Westerns: common quantification mistakes in Western blot densitometry and proposed corrective measures. BioMed Research International. 1–15. https://doi.org/10.1155/2019/5214821

Camporesi, E. 2021. Candidate biomarkers for synaptic pathology: neurogranin, neuroligins and neurexins in neurodegenerative disorders. Ph.D. dissertation, Department of Psychiatry and Neurochemistry, The University of Gothenburg, Gothenburg, Sweden.

Chubykin, A. A., Atasoy, D., Etherton, M. R., Brose, N., Kavalali, E. T., Gibson, J. R., and Südhof, T. C. 2007. Activity-Dependent Validation of Excitatory versus Inhibitory Synapses by Neuroligin-1 versus Neuroligin-2. Neuron **54**(6): 919–931. https://doi.org/10.1016/j.neuron.2007.05.029

Colón-Ramos, D. A. 2009. Chapter 2 Synapse Formation in developing Neural Circuits. Current Topics in Developmental Biology. **87**: 53–79. https://doi.org/10.1016/s0070-2153(09)01202-2

Connor, S. A., and Siddiqui, T. J. 2023. Synapse organizers as molecular codes for synaptic plasticity. Trends in Neurosciences. **46**(11): 971–985. https://doi.org/10.1016/j.tins.2023.08.001

Craig, A. M., and Kang, Y. 2007. Neurexin–neuroligin signaling in synapse development. Current Opinion in Neurobiology. **17**(1): 43–52. https://doi.org/10.1016/j.conb.2007.01.011

Dhume. H, S. 2021. Mechanisms of synapse development, plasticity and cognition of hippocampal neuronal pathways by Leucine-rich-repeat transmembrane neuronal proteins. Ph.D. dissertation, Department of Physiology and Pathophysiology, The University of Manitoba, Winnipeg, M.B.

Ducrot, C., Drouet, A., Tessier, B., Desquines, C., Cloâtre, T., Mazouzi, R., Levet, F., Favereaux, A., Letellier, M., and Thoumine, O. 2025. High-affinity detection of biotinylated endogenous neuroligin-1 at excitatory and inhibitory synapses using a tagged knock-in mouse. Proceedings of the National Academy of Sciences. **122**(22): e2411669122. https://doi.org/10.1073/pnas.2411669122.

Dunbar, J., Krawczyk, K., Leem, J., Baker, T., Fuchs, A., Georges, G., Shi, J., and Deane, C. M. 2013. SAbDab: the structural antibody database. Nucleic Acids Research, **42**(D1), D1140–D1146. https://doi.org/10.1093/nar/gkt1043

Etherton, M. R., Blaiss, C. A., Powell, C. M., and Südhof, T. C. 2009. Mouse neurexin-1α deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proceedings of the National Academy of Sciences. **106**(42): 17998–18003. https://doi.org/10.1073/pnas.0910297106

Fairless, R., Masius, H., Rohlmann, A., Heupel, K., Ahmad, M., Reissner, C., Dresbach, T., and Missler, M. 2008. Polarized Targeting of Neurexins to Synapses Is Regulated by their C-Terminal Sequences. Journal of Neuroscience. **28**(48): 12969–12981. https://doi.org/10.1523/jneurosci.5294-07.2008

Faulds, C. (n.d.). *Inside the IPI pipeline*. https://ipiproteins.shorthandstories.com/inside-the-ipi-pipeline/index.html#group-section-Antibody-discovery-afGEC9HaBC

Fuccillo, M. V., and Pak, C. 2021. Copy number variants in neurexin genes: phenotypes and mechanisms. Current Opinion in Genetics & Development. **68**: 64–70. https://doi.org/10.1016/j.gde.2021.02.010

Gera, N., Hussain, M., and Rao, B. M. 2012. Protein selection using yeast surface display. Methods. **60**(1): 15–26. https://doi.org/10.1016/j.ymeth.2012.03.014

Goda, Y., and Sabatini, B. L. 2011. Synaptic function and regulation. Current Opinion in Neurobiology. **21**(2): 205–207. https://doi.org/10.1016/j.conb.2011.03.004

Groff, K., Brown, J., and Clippinger, A. J. 2015. Modern affinity reagents: Recombinant antibodies and aptamers. Biotechnology Advances. **33**(8): 1787–1798. https://doi.org/10.1016/j.biotechadv.2015.10.004

Halber, D. 2025. *IPI targets neurexin with upcoming antibody suite*. Institute for Protein Innovation. https://proteininnovation.org/2025/02/ipi-neurexin-neuroligin-antibodies/

Han, K., and Kim, E. 2007. Synaptic adhesion molecules and PSD-95. Progress in Neurobiology. **84**(3): 263–283. https://doi.org/10.1016/j.pneurobio.2007.10.011

Harkin, L. F., Lindsay, S. J., Xu, Y., Alzu'bi, A., Ferrara, A., Gullon, E. A., James, O. G., and Clowry, G. J. (2016). Neurexins 1–3 each have a distinct pattern of expression in the early developing human cerebral cortex. Cerebral Cortex. **27**(1): 216–232. https://doi.org/10.1093/cercor/bhw394

Karagiannis, S. N., Hoffmann, R. M., Nakamura, M., Crescioli, S., Bax, H. J., Chenoweth, A., Cheung, A., Tsoka, S., Spicer, J. F., Lacy, K. E., and Thurston, D. E. 2021. Translational aspects of biologicals: monoclonal antibodies and antibody-drug conjugates as examples. In *Elsevier eBooks* (pp. 329–350). https://doi.org/10.1016/b978-0-12-820493-1.00031-3

Kim, E. 2009. Postsynaptic Development: neuronal molecular scaffolds. In *Elsevier eBooks* (pp. 817–824). https://doi.org/10.1016/b978-008045046-9.00360-0

Klatt, O., Repetto, D., Brockhaus, J., Reissner, C., Khallouqi, A. E., Rohlmann, A., Heine, M., and Missler, M. 2021. Endogenous β-neurexins on axons and within synapses show regulated dynamic behavior. Cell Reports. **35**(11): 109266. https://doi.org/10.1016/j.celrep.2021.109266

Kothari, M., Wanjari, A., Acharya, S., Karwa, V., Chavhan, R., Kumar, S., Kadu, A., and Patil, R. 2024. A Comprehensive Review of Monoclonal Antibodies in Modern Medicine: Tracing the evolution of a revolutionary therapeutic approach. Cureus. **16**(6): e61983. https://doi.org/10.7759/cureus.61983

Lee, E., Lee, J., and Kim, E. 2016. Excitation/Inhibition imbalance in animal models of autism spectrum disorders. Biological Psychiatry. **81**(10): 838–847. https://doi.org/10.1016/j.biopsych.2016.05.011

Lin, P., Chen, L. Y., Jiang, M., Trotter, J. H., Seigneur, E., and Südhof, T. C. 2023. Neurexin-2: An inhibitory neurexin that restricts excitatory synapse formation in the hippocampus. Science Advances. **9**(1): eadd8856. https://doi.org/10.1126/sciadv.add8856

Liu, X., Hua, F., Yang, D., Lin, Y., Zhang, L., Ying, J., Sheng, H., and Wang, X. 2022. Roles of neuroligins in central nervous system development: focus on glial neuroligins and neuron neuroligins. Journal of Translational Medicine. **20**(1). https://doi.org/10.1186/s12967-022-03625-y

MacConmara, M., Nwadei, I., and Kirk, A. D. 2015. Immunosuppressive biologic agents. In *Elsevier eBooks* (pp. 1343–1353). https://doi.org/10.1016/b978-1-4557-0268-8.00096-8

Missler, M., Sudhof, T. C., and Biederer, T. 2012. Synaptic cell adhesion. Cold Spring Harb Perspect Biol. **4**(4): a005694. https://doi.org/10.1101/cshperspect.a005694.

Mustafa, M. I., Alzebair, A. A., and Mohammed, A. 2024. Development of recombinant antibody by Yeast Surface Display technology. Current Research in Pharmacology and Drug Discovery. **6**:100174. https://doi.org/10.1016/j.crphar.2024.100174

Nakazawa, M., Mukumoto, M., and Miyatake, K. 2010. Production and purification of polyclonal antibodies. In Methods in molecular biology. **657**: 63–74. https://doi.org/10.1007/978-1-60761-783-9_5

Noborn, F., and Sterky, F. H. 2021. Role of neurexin heparan sulfate in the molecular assembly of synapses – expanding the neurexin code? FEBS Journal. **290**(2): 252–265. https://doi.org/10.1111/febs.1625

Padmanabhan, N., Twilley, R.E., Jones, G., Oku, S., Rabbi, M.S., Acharya, S., Basha, M.A., Rajapakse, D., Koltek, C., Ameena, N., Ahmadpour, N., Lao, Y., Guo, J., Zahedi, R.P., Ahmad, M., Hruska, M., and Siddiqui, T.J. 2025. Neurexin-1γ Drives Synaptic Transmission through Structural Integration of Neurotransmitter Release Machinery and Postsynaptic Receptor Nanodomains. Cell. [In press.]

Rabia, L. A., Desai, A. A., Jhajj, H. S., and Tessier, P. M. 2018. Understanding and overcoming trade-offs between antibody affinity, specificity, stability and solubility. Biochemical Engineering Journal. **137**: 365–374. https://doi.org/10.1016/j.bej.2018.06.003

Ramirez, A., and Arbuckle, M. R. 2016. Synaptic Plasticity: The role of learning and Unlearning in Addiction and beyond. Biological Psychiatry. **80**(9): e73–e75. https://doi.org/10.1016/j.biopsych.2016.09.002

Reverberi, R., and Reverberi, L. 2007. Factors affecting the antigen-antibody reaction. PubMed. **5**(4): 227–240. https://doi.org/10.2450/2007.0047-07

Rudenko, G. 2017. Dynamic control of synaptic adhesion and organizing molecules in synaptic plasticity. Neural Plasticity. 1–14. https://doi.org/10.1155/2017/6526151

Rudenko, G. 2019. Neurexins — versatile molecular platforms in the synaptic cleft. Current Opinion in Structural Biology. **54**:112–121. https://doi.org/10.1016/j.sbi.2019.01.009

Schrieber, L. (2010). Rheumatoid arthritis and the hand. In *Elsevier eBooks* (pp. 1–18). https://doi.org/10.1016/b978-0-7020-3377-3.00001-9

Siddiqui, T. J., and Craig, A. M. 2010. Synaptic organizing complexes. Current Opinion in Neurobiology. **21**(1): 132–143. https://doi.org/10.1016/j.conb.2010.08.016

Südhof, T. C. 2017. Synaptic neurexin complexes: a molecular code for the logic of neural circuits. Cell. **171**(4): 745–769. https://doi.org/10.1016/j.cell.2017.10.024

Südhof, T. C. 2018. Towards an understanding of synapse formation. Neuron. **100**(2): 276–293. https://doi.org/10.1016/j.neuron.2018.09.040

Taussig, M. J., Fonseca, C., and Trimmer, J. S. 2018. Antibody validation: a view from the mountains. New Biotechnology. **45**:1–8. https://doi.org/10.1016/j.nbt.2018.08.002

Vicidomini, R., Choudhury, S. D., Han, T. H., Nguyen, T. H., Nguyen, P., Opazo, F., and Serpe, M. 2024. Versatile nanobody-based approach to image, track and reconstitute functional Neurexin-1 in vivo. Nature Communications. **15**(1): 6068. https://doi.org/10.1038/s41467-024-50462-2

Wark, K. L., and Hudson, P. J. 2006. Latest technologies for the enhancement of antibody affinity. Advanced Drug Delivery Reviews. **58**(5–6): 657–670. https://doi.org/10.1016/j.addr.2006.01.025

Wener, M. H., and Mannik, M. 2004. Immune complexes. In *Elsevier eBooks* (pp. 377–399). https://doi.org/10.1016/b978-012433901-9/50017-x

Yang, P., and Mahmood, T. 2012. Western blot: Technique, theory, and trouble shooting. *North* American Journal of Medical Sciences. **4**(9): 429. https://doi.org/10.4103/1947-2714.100998

Zhang, P., Lu, H., Peixoto, R. T., Pines, M. K., Ge, Y., Oku, S., Siddiqui, T. J., Xie, Y., Wu, W., Archer-Hartmann, S., Yoshida, K., Tanaka, K. F., Aricescu, A. R., Azadi, P., Gordon, M. D., Sabatini, B. L., Wong, R. O., and Craig, A. M. 2018. Heparan Sulfate Organizes Neuronal Synapses through Neurexin Partnerships. Cell. 174(6): 1450-1464.e23. https://doi.org/10.1016/j.cell.2018.07.002

Zhou, Q. 2023. Organizing the synaptic junctions. Journal of Biological Chemistry. **299**(5). 104716. https://doi.org/10.1016/j.jbc.2023.104716