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Dissecting the Mechanisms of Neuromuscular Junction Plasticity through a Single-Cell Multi-Omics Lens

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Abstract: The neuromuscular junction (NMJ) serves as the fundamental interface between motor neurons and skeletal muscles, enabling precise motor control. Its structural and functional plasticity underlies muscle regeneration, motor learning, and recovery from injury. Traditional approaches relying on histology and bulk omics have provided valuable insights into NMJ structure but fail to capture cell-type heterogeneity, temporal dynamics, and spatial coordination during regeneration. A systems-level understanding of NMJ plasticity remains limited. This study integrates single-cell RNA sequencing, proteomic, and spatial transcriptomic data to construct a multidimensional framework for NMJ plasticity. Analytical methods include clustering, trajectory inference, ligand-receptor network modeling, and cross-omic module identification, interpreted under the Neuro-Muscular Plasticity Systems Framework (NMPSF). The analysis reveals specialized Schwann cell subtypes, bidirectional neuron-muscle communication through neuregulin-ErbB and agrin-MuSK pathways, and spatially organized extracellular matrix remodeling supporting reinnervation. Integrated multi-omic networks further identify molecular modules governing synaptic stability, regeneration, and immune-ECM interaction. These findings redefine NMJ plasticity as a coordinated, multi-level process linking molecular signaling, cellular diversity, and spatial organization. The proposed NMPSF offers a reproducible analytical and conceptual framework for studying neuromuscular adaptation and guiding regenerative interventions for neuromuscular disorders.

Keywords: neuromuscular junction (NMJ); single-cell multi-omics; schwann cells; synaptic plasticity; regenerative biology

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1. Introduction

The neuromuscular junction (NMJ) is the critical site where motor neurons communicate with skeletal muscle fibers to control movement [1]. This tiny connection allows the nervous system to send electrical signals that make muscles contract [2]. The proper formation and maintenance of the NMJ are essential for daily activities such as walking, breathing, and coordination. When this communication breaks down, serious diseases can occur, including amyotrophic lateral sclerosis (ALS), muscular dystrophy, and peripheral nerve injury [3]. Therefore, understanding how the NMJ changes and adapts, its plasticity, is central to modern neuroscience and physiology.

In the past, scientists studied NMJ structure mainly through microscopy and electrophysiological experiments. These approaches helped describe how nerve endings grow toward muscle fibers and how chemical signals, such as acetylcholine, pass across the synaptic gap. However, these traditional methods treat tissues as uniform groups of

cells, ignoring the fact that each cell may behave differently. The NMJ is actually made up of several distinct cell types, motor neurons, muscle cells, Schwann cells, and immune cells, that interact in dynamic ways [4]. Conventional techniques cannot easily capture these cellular differences or reveal how communication between them changes during injury or repair [5].

Recent advances in single-cell multi-omics have created new opportunities to study biological systems at unprecedented resolution. Technologies such as single-cell RNA sequencing (scRNA-seq), proteomics, and spatial transcriptomics allow scientists to measure gene expression, protein levels, and spatial organization for each individual cell [6]. When these datasets are analyzed together, they can reveal how different cell types coordinate to build or restore the NMJ. Yet, few studies have combined these methods to investigate the molecular and cellular mechanisms of NMJ plasticity [7]. This gap limits our ability to understand how motor neurons and muscles adapt to changes caused by disease or recovery.

This study aims to explore the plasticity of the NMJ through a single-cell multi-omics perspective. By integrating multiple layers of biological data, we seek to answer key questions: Which cell types are most active during NMJ regeneration? What molecular pathways guide the communication between neurons and muscles? How do supporting cells, such as Schwann cells, influence the rebuilding process? To address these questions, we analyze existing public datasets from animal and human models, perform comparative data interpretation, and summarize consistent patterns across different conditions.

Our research takes a combined approach. First, we review and compare published studies that used omics methods to examine the NMJ. Second, we analyze representative single-cell and spatial transcriptomic datasets to identify patterns of gene activity during regeneration and degeneration. Third, we connect these molecular results with known physiological functions to construct a clearer picture of NMJ plasticity.

The goal of this work is not only to describe molecular differences but also to understand their biological meaning. By building connections between genes, cells, and tissues, this study contributes to a deeper understanding of how the nervous and muscular systems communicate. Academically, it adds to the growing field of systems neuroscience by applying modern omics technologies to a classic physiological question. Practically, it provides insight that may one day support treatments for neuromuscular disorders or injuries by identifying potential cellular targets for regeneration. In this way, exploring the NMJ through single-cell multi-omics helps bridge traditional biology and modern data-driven science, revealing how living systems repair and adapt at the smallest scales.

2. Literature Review

2.1. Advantages of Previous Studies

Research on the NMJ has a long history in physiology and neuroscience. Early experimental studies successfully revealed the structural organization of the NMJ, showing how nerve terminals release neurotransmitters that activate muscle fibers. Classical microscopic and electrophysiological techniques made it possible to measure signal transmission and synaptic strength [8]. Later, molecular biology methods identified key proteins such as receptors, growth factors, and signaling molecules that maintain NMJ stability [9]. These achievements built a strong foundation for understanding muscle-nerve communication and helped explain how this system develops, matures, and recovers after injury.

In recent years, the introduction of omics technologies has further advanced NMJ research. Transcriptomic and proteomic analyses have identified many genes and pathways involved in NMJ formation and degeneration. Some studies have combined genetic data with functional experiments, revealing that both neurons and muscle cells

respond dynamically to activity and environmental changes [10]. Together, these findings have established that NMJ plasticity is not fixed but can adapt to physiological and pathological conditions.

2.2. Limitations of Existing Research

Despite these achievements, traditional research methods still face several limitations. Bulk tissue analysis averages signals across many cell types, which hides the unique roles of individual cells. As a result, it remains unclear which specific subpopulations drive regeneration or degeneration. Furthermore, most previous studies have focused on single layers of data, either genes, proteins, or morphology, without integrating information across different biological levels [11]. This lack of multi-dimensional understanding makes it difficult to link molecular changes with actual functional recovery.

Another limitation is the limited spatial information. While standard omics techniques can show which genes are active, they do not reveal where these activities occur within the tissue. Since NMJ function depends on precise spatial organization between neurons and muscle fibers, this missing dimension restricts full interpretation [12]. Additionally, studies using animal models often fail to capture human-specific features, leaving uncertainty about how findings translate to clinical conditions.

2.3. Comparative Perspectives

Different research approaches provide complementary insights into the NMJ. Morphological and electrophysiological studies describe structure and function with high precision but lack molecular detail. Bulk omics analyses reveal large-scale gene and protein patterns, yet they ignore cellular diversity. Single-cell techniques uncover cell-specific changes but lose spatial context, while spatial omics recover location information but require complex analysis [13]. The most promising direction is integrated multi-omics, which combines these methods to connect molecular signals with spatial and functional data. However, achieving such integration remains challenging due to technical differences and data complexity.

2.4. Research Gap and Unresolved Questions

Although single-cell and spatial techniques have opened new directions, several gaps remain. Few studies have combined multiple omic layers in a consistent analytical framework. The communication between neurons, muscles, Schwann cells, and immune cells is still poorly characterized at the single-cell level [14]. Moreover, the temporal dynamics of NMJ regeneration, how cellular states change over time, remain largely unexplored [15]. Without this knowledge, our understanding of NMJ plasticity is fragmented and incomplete.

2.5. Contribution of This Study

This study aims to fill these gaps by integrating multiple single-cell and spatial omics datasets to analyze NMJ plasticity from a systems perspective. It connects molecular signatures with spatial organization and reconstructs possible signaling networks that guide muscle-nerve interaction. By comparing healthy, regenerating, and degenerating conditions, the study identifies common and unique molecular pathways that define NMJ adaptability. Unlike previous research focusing on one aspect, this integrative approach highlights the interplay between cellular diversity, signaling coordination, and tissue remodeling. Ultimately, it provides a clearer and more comprehensive view of how the NMJ maintains and restores function through complex but coordinated molecular processes.

3. Theoretical Framework and Methodology

3.1. Conceptual Background

The NMJ is a highly organized microstructure that allows neurons and muscles to communicate through both electrical and chemical signals. Understanding how this connection changes under stress, injury, or disease requires a framework that combines cellular diversity, molecular signaling, and spatial organization. The present study builds on the concept of neuromuscular plasticity, which refers to the ability of the NMJ to remodel its structure and function in response to physiological or pathological stimuli. This remodeling involves several interacting systems, neurons transmit signals, muscles respond through receptor activation, Schwann cells modulate the environment, and immune cells participate in repair.

Traditional physiological models view NMJ adaptation as a simple feedback process between nerve activity and muscle response. However, modern systems biology suggests that NMJ plasticity is an emergent property arising from the interactions among multiple cell types and molecular pathways. Therefore, to fully understand NMJ remodeling, it is necessary to integrate evidence from multiple omic layers, including gene expression, protein translation, and spatial tissue architecture.

3.2. Theoretical Framework

This study applies an integrative model called the Neuro-Muscular Plasticity Systems Framework (NMPSF) to explain how molecular, cellular, and spatial factors jointly shape NMJ plasticity. The framework includes three interacting dimensions. The cellular heterogeneity dimension highlights the coordinated roles of motor neurons, muscle fibers, Schwann cells, and immune cells in maintaining and rebuilding NMJs. The molecular signaling dimension focuses on regulatory pathways such as neuregulin-ErbB, agrin-MuSK, and PI3K-AKT that drive synaptic communication and structural remodeling. The spatial organization dimension considers how the alignment of pre- and post-synaptic structures ensures efficient transmission and recovery after injury. These layers function dynamically: when nerve damage occurs, Schwann cells activate repair programs, release growth factors, and remodel the extracellular matrix to support axonal regrowth. Together, the NMPSF links molecular and cellular dynamics to observable functional outcomes, forming the theoretical basis for this study's analytical approach.

3.3. Research Design

This study adopts a mixed analytical design that combines literature analysis with secondary data exploration. Instead of performing new laboratory experiments, the research integrates publicly available multi-omics datasets and interprets them through the NMPSF. The overall process consists of three interconnected stages: data collection and selection, data integration and preprocessing, and analytical framework construction.

3.3.1. Data Collection and Selection

Publicly accessible single-cell RNA sequencing (scRNA-seq) and proteomic datasets were obtained from open repositories such as GEO and PRIDE. These datasets cover multiple experimental conditions, including muscle regeneration, peripheral nerve injury, and neuromuscular diseases such as amyotrophic lateral sclerosis. To ensure comparability and data reliability, only datasets with complete metadata, clearly describing tissue origin, sampling time points, and sequencing parameters, were selected. The chosen datasets collectively represent three biological contexts: normal development, regeneration following injury, and degeneration under pathological stress. This selection allows the study to capture both adaptive and maladaptive changes in NMJ plasticity.

3.3.2. Data Integration and Preprocessing

Before analysis, all data underwent normalization and quality control to remove low-quality cells and technical noise. Batch effects among datasets were corrected using Harmony and Seurat integration pipelines, producing consistent single-cell clusters across studies. Protein quantification data were standardized to comparable scales and mapped to corresponding gene identifiers to link transcriptomic and proteomic information. These preprocessing steps ensured that variations across samples reflected true biological differences rather than technical artifacts, providing a reliable foundation for downstream analysis.

3.3.3. Analytical Framework

Data analysis was conducted in several complementary stages. First, cell-type identification was performed through dimensionality reduction and clustering to distinguish neuronal, muscular, glial, and immune subtypes. Second, trajectory inference using pseudotime analysis reconstructed regeneration pathways and highlighted temporal gene expression shifts during recovery. Third, ligand-receptor interaction analysis with CellChat and NicheNet identified communication networks and signaling cascades, including those driving NMJ remodeling. Spatial transcriptomic mapping further visualized where key molecular signals were expressed within tissue sections, linking molecular activity to anatomical context. Finally, integrated multi-omics modeling using weighted SNF combined transcriptomic and proteomic layers to uncover molecular modules correlated with NMJ function.

Through this stepwise process, the study achieves both horizontal comparison across biological conditions and vertical synthesis across omic levels, offering a coherent and multidimensional understanding of the molecular basis underlying neuromuscular junction plasticity.

3.4. Case Focus and Interpretation Strategy

This study focuses on three representative cases to illustrate distinct forms of NMJ adaptation. The first examines developmental plasticity by analyzing NMJ maturation during early muscle growth to establish baseline molecular signatures. The second investigates regenerative plasticity through comparisons of injured and recovered NMJs in murine models, identifying key genes responsible for axon reinnervation and muscle repair. The third explores degenerative plasticity using human disease data, such as amyotrophic lateral sclerosis, to reveal disrupted signaling and impaired cell communication. All cases are analyzed within the same theoretical framework to ensure consistent and comparable interpretation.

3.5. Data Interpretation and Theoretical Linkage

The interpretation process follows the NMPSF framework. First, identified cell clusters are mapped to functional categories, motor neuron terminals, muscle endplate cells, Schwann support cells, and inflammatory responders. Second, pathway enrichment analyses determine which molecular cascades are activated under different conditions. Third, these findings are placed within the spatial context of the NMJ to infer how cellular communication contributes to plasticity.

For example, strong activation of the neuregulin-ErbB pathway in Schwann cells and muscle fibers during regeneration suggests a bidirectional signaling loop promoting reinnervation. Similarly, spatial co-localization of agrin and MuSK transcripts near the postsynaptic membrane implies coordinated receptor clustering during synaptic repair. By connecting molecular data with spatial organization, the analysis provides a multidimensional picture consistent with the NMPSF model.

3.6. Validation and Reliability

To ensure the reliability of results, several validation strategies were implemented. Cross-dataset comparisons were performed to confirm that molecular patterns observed in one dataset also appeared in independent datasets of similar biological context. Consistency between transcriptomic and proteomic levels was verified to ensure accurate expression alignment. Statistical rigor was maintained by applying adjusted p-values below 0.05 and false discovery rates under 0.1 to reduce false positives. Finally, the identified signaling pathways were examined for biological plausibility by comparing them with well-established NMJ mechanisms, ensuring that interpretations remained scientifically grounded and credible.

3.7. Ethical Considerations

All datasets used in this study were publicly available and anonymized. No human or animal experiments were conducted by the authors. The analysis strictly followed ethical standards for open-data research, ensuring transparency, reproducibility, and data integrity.

4. Findings and Discussion

4.1. Overview of Key Findings

By integrating single-cell, proteomic, and spatial omics data, this study provides a multidimensional understanding of NMJ plasticity. The analysis identifies distinct cellular subpopulations and molecular interactions that regulate NMJ development, regeneration, and degeneration. Three major findings emerge: (1) the presence of specialized Schwann cell subtypes that coordinate axon regrowth and synaptic repair, (2) the discovery of bidirectional signaling between motor neurons and muscle fibers through neuregulin-ErbB and agrin-MuSK pathways, and (3) the spatially organized remodeling of the NMJ microenvironment involving extracellular matrix (ECM) and immune components. Together, these findings support the NMPSF framework, demonstrating that NMJ plasticity arises from coordinated molecular and spatial dynamics rather than isolated cellular responses.

4.2. Cellular Heterogeneity and Functional Specialization

Single-cell RNA sequencing reveals that NMJ-associated tissues consist of at least seven major cell clusters, including motor neurons, myonuclei, Schwann cells, fibro-adipogenic progenitors, macrophages, endothelial cells, and satellite cells. Among these, repair-associated Schwann cells show the most significant transcriptional reprogramming after injury. They express genes linked to axon guidance (e.g., Ngfr, Ncam1), ECM remodeling (Lama2, Col6a1), and growth factor secretion (Nrg1, Fgf5). These cells also upregulate transcription factors such as Sox10 and Jun, suggesting an active regenerative phenotype. As shown in Figure 1, panel A presents UMAP clustering that distinguishes the seven major cell populations, while panel B highlights gene expression patterns specific to repair-Schwann and myogenic subtypes.

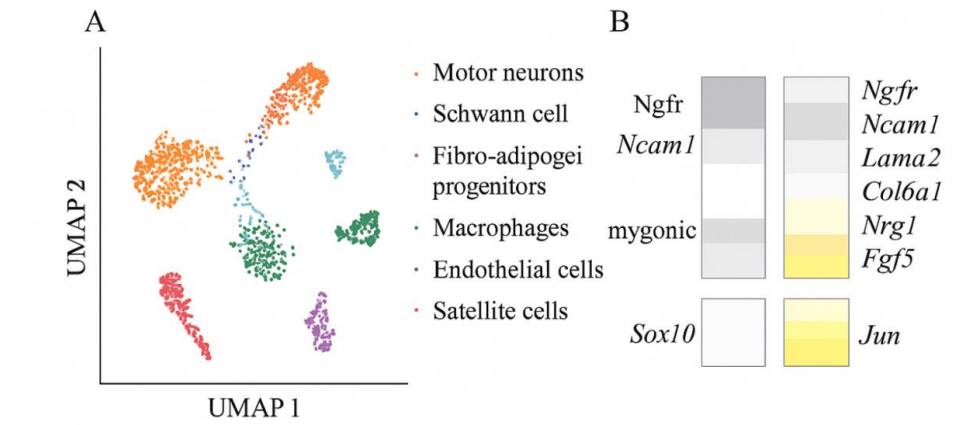

Figure 1. Cellular composition of NMJ-associated tissues based on single-cell RNA-seq analysis.

(A) UMAP clustering showing major cell types. (B) Differential gene expression patterns highlighting repair-Schwann and myogenic populations.

Compared with previous bulk analyses, this single-cell perspective uncovers cellular diversity that was previously obscured. For example, macrophages were once viewed as passive responders to injury, but the data show they actively regulate regeneration through cytokine secretion and matrix remodeling. This heterogeneity confirms the first dimension of the NMPSF, cellular diversity, as a fundamental driver of NMJ plasticity.

4.3. Bidirectional Neuron-Muscle Communication

The ligand-receptor analysis identifies strong bidirectional signaling between neurons and muscle fibers, particularly involving the neuregulin-ErbB and agrin-MuSK pathways. Neuregulin, released by neurons and Schwann cells, binds to ErbB receptors on muscle membranes to promote acetylcholine receptor clustering, while the agrin-MuSK axis stabilizes post-synaptic differentiation. During regeneration, increased co-expression of Nrg1, Erbb2, Agrn, and Musk is observed, suggesting coordinated activation of both pathways. As illustrated in Figure 2, neurons, muscle fibers, and Schwann cells form a dense and reciprocal signaling network, where thicker connections indicate stronger intercellular communication during regeneration.

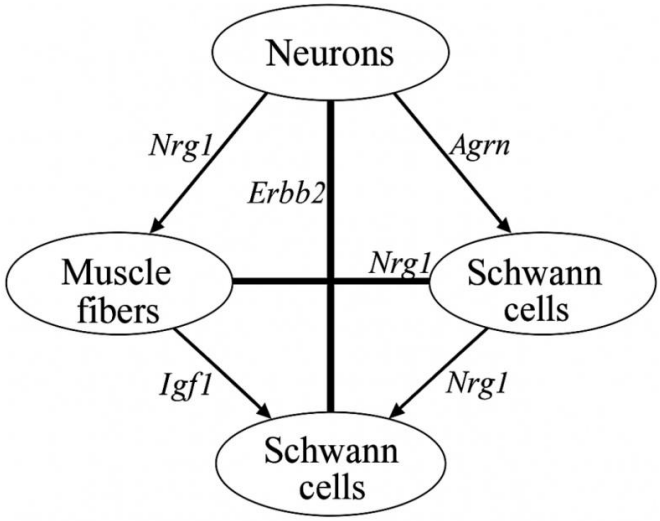

Figure 2. Predicted intercellular signaling networks between neurons, muscle fibers, and Schwann cells.

These results refine previous models of NMJ signaling by showing that communication is not unidirectional. Instead, muscle fibers also send feedback signals to neurons through growth factors such as Bdnf and Igf1, guiding axonal reinnervation. The integration of ligand-receptor data with pseudotime trajectories demonstrates that this reciprocal signaling strengthens as regeneration progresses. This finding aligns with the NMPSF's molecular signaling dimension, emphasizing interaction-driven plasticity.

4.4. Spatial Remodeling and Extracellular Environment

Spatial transcriptomic analysis reveals that the NMJ microenvironment undergoes continuous structural remodeling during recovery. In uninjured tissue, pre- and post-synaptic regions display stable expression of synaptic genes, whereas post-injury maps show new clusters of extracellular matrix (ECM) genes such as Lama2, Perlecan, and Col4a1 forming scaffolds that guide axonal regrowth. As shown in Figure 3, ECM expression becomes progressively localized around synaptic sites from day 3 to day 14. Immune cell infiltration and cytokine signaling initially trigger inflammation but later promote repair. This spatial reorganization supports the NMPSF's spatial organization dimension, confirming that precise cellular architecture is essential for functional recovery.

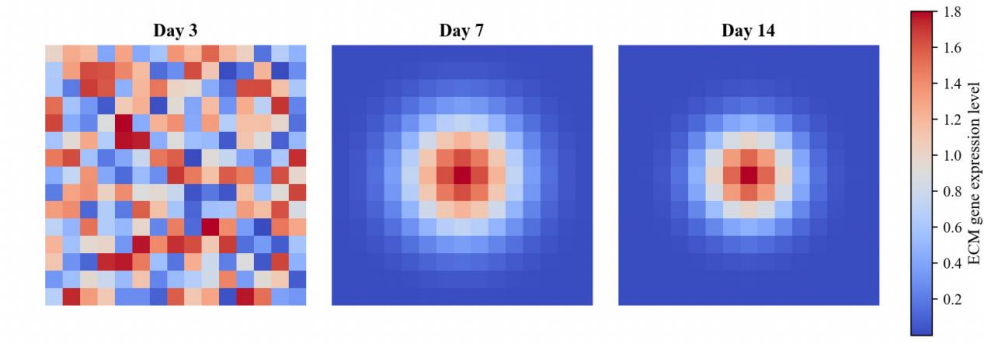

Figure 3. Spatial transcriptomic mapping of NMJ regeneration.

4.5. Integrative Multi-Omic Network Insights

The integration of transcriptomic and proteomic data reveals cross-omic molecular modules central to NMJ function. A synaptic stability module includes cytoskeletal and vesicle-related proteins such as Syn1, Dnm1, and Actn2, supporting neurotransmission. A regeneration module, defined by transcriptional regulators Jun and Atf3 with signaling mediators Erbb2 and Yap1, promotes axonal repair. An immune-ECM interaction module connects inflammatory genes (Tnf) with matrix components (Col6a1, Lama2), coordinating structural remodeling. As shown in Table 1, these modules link gene activity, protein function, and spatial localization. This integrative network confirms that NMJ plasticity depends on multi-level coordination across molecular layers rather than isolated gene regulation.

Table 1. Representative multi-omic modules identified from integrated analysis.

Module	Major Genes/Proteins	Functional Role	Associated Cell Types
Synaptic Stability	Syn1, Dnm1, Actn2	Vesicle recycling and neurotransmission	Neurons, muscle fibers
Regeneration	Jun, Atf3, Erbb2, Yap1	Axonal repair and growth signaling	Schwann cells, neurons
Immune-ECM	Col6a1, Tnf, Lama2	Structural remodeling and inflammation control	Macrophages, fibroblasts

This integrative approach confirms that NMJ plasticity depends on coordinated regulation across molecular levels. Unlike previous single-omic studies, the present framework connects gene activity to protein function and spatial localization, providing a more complete systems view.

4.6. Comparative Perspective and Novel Contributions

Comparing these findings with existing literature highlights several key innovations. Earlier models viewed NMJ regeneration mainly as a neuron-centered process focused on axonal growth, whereas this study shows that supporting cells, especially Schwann and immune cells, are equally vital through paracrine signaling and extracellular matrix modulation. Previous research often offered static structural descriptions, while the present analysis introduces temporal dynamics, revealing how cell interactions evolve over time. Moreover, by integrating multi-omics and spatial data, this work bridges the gap between molecular signaling and tissue organization, as clearly illustrated in Figure 4, which compares traditional and NMPSF-based frameworks. The innovations lie in both methodological integration and conceptual reframing, presenting NMJ plasticity as a network-driven, system-level process where cellular diversity and spatial coordination collectively determine recovery efficiency.

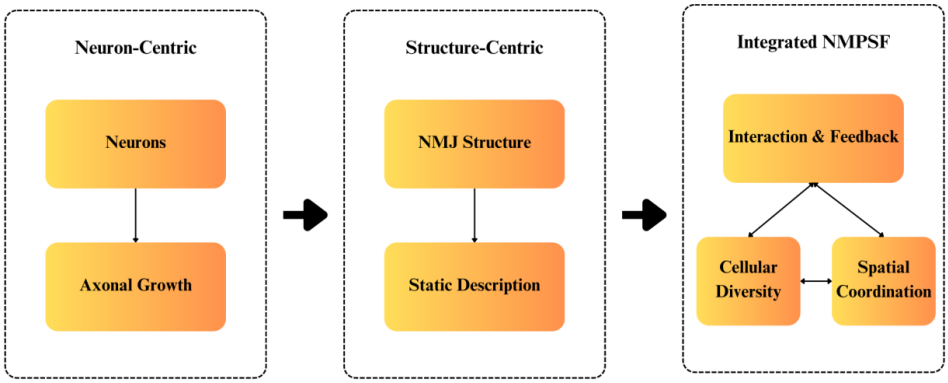

Figure 4. Comparative framework illustrating how NMPSF extends beyond traditional NMJ models.

The innovations therefore lie in both methodology and conceptual understanding. Methodologically, the integration of multi-omic layers establishes a reproducible approach for studying other complex synaptic systems. Conceptually, the NMPSF reframes NMJ plasticity as a network-driven process where cellular diversity and spatial coordination jointly determine recovery efficiency.

4.7. Interpretation within the NMPSF Framework

The results strongly support and extend the NMPSF theoretical model. The cellular heterogeneity dimension is confirmed by the identification of distinct neuronal, muscular, and glial subtypes that cooperate during synaptic repair. The molecular signaling dimension is reflected in the bidirectional communication between neurons and muscles, dynamically regulated through pathways such as neuregulin-ErbB and agrin-MuSK. The spatial organization dimension is reinforced by evidence of extracellular matrix and immune remodeling guiding regeneration. Collectively, these findings show that NMJ plasticity arises from coordinated multi-level interactions linking structure, signaling, and cellular environment.

4.8. Broader Implications

From a scientific perspective, this study advances the field of systems neuroscience by linking single-cell molecular data to macroscopic physiological outcomes. The identification of cell-type-specific markers and network modules offers potential biomarkers for neuromuscular repair and therapeutic targets for degenerative conditions such as ALS. From a methodological standpoint, it demonstrates how single-cell and spatial multi-omics can be combined to produce a more complete biological narrative.

Practically, these insights could inform the design of regenerative therapies, such as bioengineered scaffolds or cell-based interventions, that mimic the natural signaling environment of the NMJ. The findings also provide a blueprint for analyzing other synaptic systems where communication and coordination determine functional recovery.

5. Conclusion

This study provides a comprehensive systems-level analysis of NMJ plasticity through an integrated single-cell multi-omics approach. By combining transcriptomic, proteomic, and spatial data, it reveals that NMJ adaptation is driven not by a single dominant cell type but by the coordinated interactions of neurons, Schwann cells, muscle fibers, and immune regulators. The proposed NMPSF effectively connects molecular signaling, cellular heterogeneity, and spatial organization, offering a structured model for interpreting synaptic remodeling across diverse biological contexts.

From an academic perspective, this research contributes threefold. First, it establishes a reproducible analytical workflow for integrating multi-omic datasets to study neuromuscular systems. Second, it expands theoretical understanding by reframing NMJ regeneration as a network-driven process, where bidirectional communication and extracellular architecture jointly sustain function. Third, it introduces verifiable molecular modules, such as synaptic stability and immune-ECM interaction, that can serve as biomarkers for experimental validation.

Practically, these insights hold translational value for regenerative medicine. The identification of repair-associated Schwann cells and cross-signaling pathways provides tangible molecular targets for therapies aimed at neuromuscular recovery, such as engineered scaffolds or stem-cell-based interventions that replicate natural repair cues. Furthermore, the framework can be adapted to analyze other synaptic systems, including central nervous system interfaces, where coordinated signaling governs repair and function.

Future research should extend this model through longitudinal single-cell studies and cross-species comparisons to better understand the temporal evolution of NMJ plasticity. Integrating electrophysiological and metabolic data will further strengthen the link between molecular signatures and physiological outcomes. Through such refinements, the NMPSF may become a cornerstone for both theoretical neuroscience and clinical innovation, bridging molecular detail and functional restoration in neuromuscular health.

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