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Neuroimaging Techniques in Diagnosing and Understanding Neuromuscular Junction Disorders

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Abstract

Neuromuscular junction (NMJ) disorders, which impaired communication between motor neuron and skeletal muscle, leading to muscle weakness and paralysis. Accurate diagnosis of these conditions remains challenging due to their varied etiology and clinical presentation. This article focuses on the role of neuroimaging in enhancing the diagnosis and understanding of NMJ disorders. Advances in imaging technologies, including magnetic resonance imaging (MRI), computed tomography (CT), and functional MRI (fMRI), provide detailed insights into the structure and function of NMJs. Allowing for improved identification of pathological changes. These image modalities offer non-invasive approaches that complement traditional diagnostic techniques, enabling earlier and more precise detection of NMJs disorders. The integration of these advanced methods into clinical practice improves patient outcomes by facilitating timely and accurate diagnosis. Despite the progress in neuroimaging, challenging remain, particularly in standardization and interpretation of results across different techniques. Future developments in imaging technology are expected to overcome these hurdles, paving the way for more effective diagnosis and treatment of NMJ disorders.

Keyword: Neuromuscular junction disorders; NMJ disorders; fMRI, CT, neuroimaging; neurological imaging,

1. Introduction to Neuromuscular Junction Disorders

Neuromuscular junction (NMJ) disorders are a diverse group of diseases that affect the synapses between motor neurons and skeletal muscle, leading to muscle weakness or paralysis (Zou & Pan, 2022). These disorders can be classified based on different criteria, such as the pathophysiology (post-synaptic, pre-synaptic, or non-synaptic), the type of immune response (antibody-mediated or complement-mediated), or the localization of pathology (central nervous system, peripheral nervous system, or NMJ) (Nemeth et al., 2024). Each type of NMJ disorder has unique clinical features, prognosis, and treatment options. For example, autoimmune NMJ disorders often cause fluctuating muscle weakness, while hereditary NMJ disorders are usually associated with muscle wasting and contractures. (Zhu et al., 2023)

NMJ disorders have a low prevalence and incidence compared to other neurological diseases, but some autoimmune NMJ disorders are common and can significantly affect the quality of life of patients. (Verschuuren et al.2022) Myasthenia gravis is the most prevalent NMJ disorder, with an estimated prevalence of 14-179 cases per million population and an annual incidence of 5-12 cases per million population. (Mevius et al.2023) NMJ disorders can occur at any age but often debut in middle adulthood or early childhood, depending on the type of NMJ disorder. Most NMJ disorders are chronic progressive diseases, affect the daily life of patients by impairing physical activities such as walking, swallowing, or breathing, and by causing fatigue (Mejia Maza et al., 2021).

1.1. Explanation and Categorization

Disorders of the neuromuscular junction (NMJ) impact the communication between nerves and muscles, causing challenges with movement, coordination, breathing, and swallowing. Symptoms may include weakness, muscle loss, fatigue, or a sense of heaviness in the limbs. (Huijbers et al.2022) Most NMJ disorders are autoimmune or inherited. Autoimmune NMJ conditions can be caused by the body developing antibodies against a specific receptor or protein. The most common autoimmune NMJ disorder is Myasthenia Gravis (MG), where the body produces antibodies against the acetylcholine receptor (AChR). (Borges & Richman, 2020) The NMJ is a highly specialized synapse between a motor neuron and muscle fiber. Here, the motor neuron terminal releases the neurotransmitter acetylcholine (ACh), which binds to nicotinic ACh receptors, leading to muscle excitation. To ensure proper signal transmissions, several proteins are present in the pre- and postsynaptic compartments of NMJs.

Mutations affecting proteins associated with NMJ function may manifest as congenital myasthenic syndrome, congenital muscular dystrophy, or spinal muscular atrophy (Nemeth et al., 2024).

Myasthenic syndromes are conditions characterized by reduced muscle excitability caused by dysfunction at the NMJ, either due to autoimmune factors, genetic mutations, or environmental conditions (Kaler et al., 2020). Genetic forms of myasthenic syndromes are congenital illnesses caused by mutations in the genes for NMJ proteins, where around 30 different genes have been associated with similar clinical presentations. (Cremers et al.2020) The NMJ represents a dynamic and highly organized postsynaptic specialization that may expand and retreat depending on the availability of motoneuron input and activity. It has been used as a model to study cellular mechanisms that underlie the development, maintenance, and reorganization of synapses. Furthermore, alterations in NMJs are observed in neuromuscular disorders, including those associated with motoneurons and myopathy (Mejia et al.2021). Recent advances in neuroimaging techniques have opened the possibility of non-invasive exploration of NMJs in high temporal and spatial resolution.There are currently no studies that demonstrate the potential of neuroimaging techniques in the diagnosis and understanding of NMJ disorders.

1.2. Epidemiology and Impact

The prevalence of neuromuscular junction (NMJ) disorders is not fully understood because population-wide data about disease incidence, assessed using broadly applicable criteria, is lacking (Mejia Maza et al., 2021). A broad mix of pathophysiological mechanisms leads to distinct types of NMJ disorders, broadly categorized into presynaptic, postsynaptic, and structural pathology, which in turn leads to either neuromuscular transmission defects or NMJ instability. Potential target populations include patients who present with fatigable muscle weakness, myogenic MUDE or AXDE on high-density EMG, patients with tests suggestive of structural pathology at the NMJ (EMG-jitter, E-MG), and patients with myogenic MUDE on needle EMG. (Preston & Shapiro, 2020)

As a result, the notion of "minimally invasive" is subjective in a disease group with a wide variety of diagnostic tests (some invasive), and the disease types included in the target population are so diverse that one single uni-modal test hardly can address them all (J. Strijkers et al., 2019). The number of patients that could be reached is highly sensitive to the methodology chosen, ranging from a few hundred patients potentially accrued if only patients with a classical MG phenotype (graminaceous postsynaptic NMJ disorder) to tens of thousands being applicable if all types of NMJ disorders would be considered. This highlights the need for epidemiological studies estimating the yield of existing tests for each type of NMJ disorder in separate and unselected cohorts, and potentially myopathy and neuropathy controls. One of the points of note in the proposed approach is that all tests are deemed to be initially done outside of the target patient cohort against which validation is performed (although as mentioned above, some overlapping patients may be involved).

2. Neuroimaging in Clinical Practice

The broader role neuroimaging plays in clinical practice is rapidly increasing and finds specific relevance in the diagnosis and understanding of neuromuscular junction (NMJ) disorders. Therefore, a description of neuroimaging techniques, from older myography and ultrasound approaches through to the most recent developments in MRI and PET scans, will be provided here. This will follow the scientific principles behind these approaches as they relate to NMJ investigations (Minnerop et al., 2018). It is hoped that these new approaches can be integrated into the standard workup of NMJ diseases as prescreening or alternative techniques to patients with a clinical suspicion of such diseases.

2.1. Expanding the Vital Role of Neuroimaging in Diagnosing Neuromuscular Junction Disorders

Neuroimaging techniques play a crucial role in the diagnosis and understanding of neuromuscular junction (NMJ) disorders. NMJs are essential for motor control, relaying signals from motor neurons to muscles. Despite the importance of NMJ disorders, their study using non-invasive techniques has been limited. However, advancements in optical techniques and functional imaging in specific deep tissues have made it possible to perform bioimaging in live animal models. Early studies used purified toxins and markers for visualization, but the impact of neuroimaging techniques in the clinical context is more recent and underexplored (Mejia Maza et al., 2021). Here, focus is placed on neuroimaging techniques currently in use and being prototyped clinically to study NMJ disorders.

NMJ disorders manifest as weakness or fatigability in an episodic manner (Cantó-Santos et al., 2020). Weakness may occur in specific muscle groups or be generalized. Ocular muscles are often affected, leading to diplopia and tired eyes. Bulbar symptoms may also occur, such as slurred speech and difficulty swallowing. Weakness usually improves after rest (Nemeth et al., 2024). Examination of voluntary movements can reveal the insights of NMJ transmission. Symptoms can be elicited by electrophysiological studies to show decremental response on repetitive stimulation. High-frequency repetitive stimulation with a more than 10 Hz frequency can provoke a greater action potential (increased jitter and blocking) (Wang et al.2023). Performing these provocative

tests on all NMJ disorders takes time or effort, particularly in children. Hence, the initial examination often includes non-invasive imaging studies.

2.2. Common Neuroimaging Techniques

Neuroimaging techniques play a crucial role in the assessment of normal and diseased conditions of the central and peripheral nervous systems. Many neuromuscular junction (NMJ) disorders are congenital or genetic in origin. Thus, discovering genes responsible for NMJ disorders has opened opportunities for new diagnostic tools and knowledge of bilateral involvement of NMJ disorders using neuroimaging (Mejia Maza et al., 2021). Recently developed neuroimaging techniques used in the assessment of the NMJ, with an emphasis on those employed in humans. Choices regarded essential design elements that all imaging modalities must manage to quantify NMJ structures and function. A review of exciting developments in molecular neuroimaging relates to the NMJ and muscle and their role within the range of the NMJ has been considered (J. Strijkers et al., 2019). Common neuroimaging techniques in animal studies are presented. An emphasis is placed on imaging at spatial and time scales relevant to NMJ disorders. Future strategies and hurdles for advancing the field are explored.

Common techniques used for neuroimaging include optical imaging, magnetic resonance imaging (MRI), electroencephalography (EEG), magnetoencephalography (Rogers et al.), computed tomography (CT), and positron emission tomography (PET).

3. Principles of Neuroimaging

Neuroimaging comprises diagnostic strategies that take advantage of the body's anatomical and physiological properties, allowing for the assessment of the central and peripheral nervous system pathologies without the need for invasive procedures (Minnerop et al., 2018). The widely used neuroimaging techniques are magnetic resonance imaging (MRI) and computed tomography (CT), which provide valuable data about the structure of the nervous system through anatomical images. This knowledge about neuroimaging techniques is fundamental for understanding their subsequent application (Bodea & Westmeyer, 2021)

Both CT and MRI share the same physical principle, namely the interaction of ions with electromagnetic waves (Caspani et al., 2020)CT makes use of X-rays, which have a very high energy level and may cause several side effects (Withers et al.2021). MRI, on the other hand, is based on the interaction of low energy waves (radiofrequency) with atomic nuclei in a strong magnetic field and does not generate ionizing radiations. MRI has different contrasts which depend on the type of atomic nuclei being probed (1H, 13C, 31P…), the surrounding environment, and the parameters used during signal acquisition (Gaeta et al.2021). But it is the chemical environment that allows for the specific character of biomedical imaging. In particular, the contrast generated through proton magnetic resonance imaging (1H-MRI) depends on the cellular and molecular biophysical properties of tissues such as their water fraction and cellular architecture (J. Strijkers et al., 2019). Therefore, every kind of tissue has a specific MR spectrum that can be used to identify them.

3.1. Basic Principles of Magnetic Resonance Imaging (MRI)

Motor neurons proliferate from the neural tube during the embryonic developmental stage in mammalians, and they elongate axons to target muscle fibers. By the 13th prenatal day (P13) few times embryonically detectable Olivo-Cerebellar (OC) fibers become re-detectable by 28 postnatal days and detectable in the visible polymer matrix (JP–Glimmer) model of neocortical femtosecond pulse plasma imaging heralding a long forgotten neuronal path (Shaker et al.2021). Before P13 the OC fibers are not visibly detectable blue meta luminescent with the femtosecond pulse plasma imaging method. This system forms semi-closed triangular body involving and glutamatergic synapsing with contralateral climbing fibers and crossing the anterior midline while releasing serotonin, substance P, and neuropeptide Y before P28. Further thicker myelinated fibers approach and faintly lace the growing dendrite recruitment during the construction of synapses, and the activation of OC fibers is crucially involved in the initiation and execution of cerebellar learning of conditioned eye blink and motor timing (Chen et al., 2019).

3.2. Basic Principles of Computed Tomography (CT)

CT and MRI imaging are both very popular biomedical imaging techniques today. They allow non-invasive in vivo imaging of anatomical and pathological features with very high accuracy (Minnerop et al., 2018). CT uses X-ray attenuation of different tissues to create good density resolution images or 3D models of internal structures. Cross-sectional or 2D images can then be computed from 3D volumetric data. The CT imaging systems consist of an X-ray source and an array of detectors, located on opposite points to each other across the scanned object. The basic principle of CT imaging consists of rotating the X-ray source/detector assembly around the object and linearly moving them along (Withers et al.2021). During this process, a number of view angles of projection images are accumulated, which reflects the attenuation information at those angles. The projection data is then used to reconstruct the internal structures of the scanned object by employing a tomographic reconstruction algorithm such

as the filtered back-projection (FBP) algorithm. The mathematical basis of that approach is the Radon transform and the Fourier transform. CT is popular for its accurate depiction of bone structures, while MRI is better for soft tissues like muscles and fat. Nevertheless, their complementary image quality has encouraged researchers and scientists to develop numerous methodologies to ensure a successful joint use of both techniques (Gargiulo et al., 2014).

4. Neuroimaging Modalities for Neuromuscular Junction Disorders

Neuroimaging strategies may help delineate the anatomic, pathologic, or physiologic substrate involved in NMJ diseases and help differentiate mimics. The advent of better dedicated imaging tools, high resolution techniques, and new contrast agents have contributed to improved detection, visualization, and evaluation of the muscle (Hok et al., 2021). MRI has previously been shown to be sensitive to various neuromuscular diseases. The literature review provides technical aspects, imaging characteristics, pitfalls, differential diagnoses, and prognostic indicators of NMJ disorders. Most of the literature regarding various physiologic imaging techniques centers on the use of SPECT/PET in NMJ diseases.

A somatosensory evoked potential and transcranial magnetic stimulation study conducted in neuropathic patients with peripheral vascular disease comparing different electrophysiological approaches against MRI findings suggests that MRI abnormalities correlate with motor dysfunction (Wilkinson et al.2020). This is in support of the application of brain MRI in the differential diagnosis of peripheral neuropathy (e.g., in patients with demyelinating hereditary neuropathies). Neuromuscular patients undergoing routine MRI were found to have incidental CNS findings such as intracranial hypertension, Chiari malformation, and vascular malformations (Moncho et al.2023). The independent effect of these incidental findings on treatment modification was such that 27% of patients needed further investigation prior to muscle biopsy. In younger children with an unclear neuromuscular disease, the planned work-up was significantly changed in cases with known CNS malformations (e.g., pontocerebellar hypoplasia or a disorder of glycosylation) (Horber et al.2021).

4.1. Magnetic Resonance Imaging (MRI) in Neuromuscular Junction Disorders

Magnetic resonance imaging (MRI) has been widely adopted as a complementary tool for the assessment of neuromuscular junction (NMJ) disorders. MRI is rarely abnormal in this disorder. However, normal MRI does not rule out NMJ disorders (Punga et al.2022), particularly in younger patients or those with particularly mild muscle involvement. It finds generalized muscle fatty replacement in slowly progressive forms, like myotonic dystrophy type 1. MRI, combined with electrophysiology or biochemistry, aids in classifying broader muscle dystrophies. MRI findings also suggest a distinct form of amyoplasia congenital arthrogryposis multiplex, specifically the pleomorphic variant with dystrophic changes and hyperintensity on T2-weighted images (Chen et al., 2019). MRI is a new research direction for neuromuscular diseases and is worth exploring further.

There are two forms of MRIs: chemography and spectroscopy. Chemography focuses on studying simple molecular chromation and compounds containing hydrogen, nitrogen, phosphorus, and other elements (Wieczorek et al., 2022). Spectroscopy emphasizes the biomagnetic effects of studying high-field superconductors and intrinsic diamagnetic materials with diamagnetic characteristics in spectroscopy applications (Sharma, 2021). In this premium series, the evaluation perspective of myocardial damage level via transmural injury spectrum correlated with the degree of clinical symptoms of myocardial ischemia is presented (Martinez et al.2021). Additionally, myocardial classification through conductivity inhomogeneity is also discussed. Chemo-physical proactive MRI approaches for the spectroscopic study of metallo-porphyrins complexes are expounded in a secondary series. These MRI/biomagnetic properties of molecular complexes can be further used for the selective detection of biocyclization chemical following endo- and exohedric paths in bio-related systems for mediated anticancer therapies (J. Strijkers et al., 2019).

4.2. Computed Tomography (CT) in Neuromuscular Junction Disorders

Computed tomography (CT) is another important imaging modality, which has great potential in the investigation of muscle diseases. CT and CT-based 3D modeling techniques have been successfully adopted to assess, quantitate and characterize the progression of atrophy in human muscle subsequent to permanent lower motor neuron (LMN) denervation (Gargiulo et al., 2014). They have also been applied to quantify muscle recovery induced by functional electrical stimulation (FES) as well as to assess and characterize bone quality in patients undergoing total hip replacement (Carson & Buick, 2021). Such applications are relevant to neuromuscular junction (NMJ) diseases due to their underlying pathology of muscle denervation and atrophy. Detailed protocols of CT imaging and processing for muscle applications were outlined.

CT data and appropriate segmentation techniques have been applied to adequately quantify changes in muscle density and composition by associating Hounsfield unit values with different colors (Huber et al.2020). This method could provide valuable diagnostic and follow-up information on injured or diseased human muscle. The

same methodology could also be applied in the evaluation of muscle and bone conditions in patients with NMJ disorders, providing their quantitative assessment and, possibly, early diagnosis (Minnerop et al., 2018).

5. Advanced Neuroimaging Methodologies for Enhanced Data Acquisition

5.1. Diffusion Tensor Imaging (DTI)

Diffusion tensor imaging (DTI) is an advanced magnetic resonance imaging (MRI) technique that provides in vivo assessment of the directionality and integrity of the brain's white matter tracts, i.e., the pathways for communication between different brain areas (Palacios et al.2022). DTI assesses diffusion of water molecules in tissues, characterizing white matter microstructure in voxel-wise maps of three scalar indices: (i) apparent diffusion coefficient (ADC), (ii) fractional anisotropy (FA), and (iii) eigenvalues λ1,2,3. The diffusion of water in white matter is highly anisotropic because of the axonal membranes and myelin sheaths. DTI therefore has good spatial resolution and is sensitive to pathologies that disrupt the intactness of these tracts, such as those observed in neuromuscular junction (NMJ) disorders (E. Rizea et al., 2012).

DTI is a well-established technique used in several studies, which examined changes in the brain's white matter in patients with neuromuscular disorders, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) (Ahn & Lee, 2011). Both disorders are characterized by degeneration of motor neurons in the anterior horn of the spine and/or the motor cortex and selection of fibers within the corticospinal tract (Fullam & Statland, 2021) In addition to the lower motor neuron degeneration in the spinal cord or brain stem, the motor neurons in the cortical layer are up-regulated and contribute to a dying-back mechanism and their secondary degeneration (La et al.2021). Importantly, changes in the brain microstructure underlying the neurodegenerative mechanisms are non-invasive, symmetrical between both hemispheres (Sanches et al.2021), and caudorostral (affecting the pyramidal tract at the sites of the corona radiata and internal capsule; biases fibers of large diameter).

5.2. Functional MRI (fMRI)

Functional MRI (fMRI) is a non-invasive technique used to detect regional changes in brain activity by monitoring the blood-oxygen-level-dependent (BOLD) signal: the fMRI signal increases in regions of the brain that receive more blood flow, as these regions have higher metabolic demands (Sebastiano et al.2020). Its three-dimensional images allow for the investigation of various neurological disorders, including neurodegenerative diseases. In conjunction with other neuroimaging modalities such as structural MRI and DTI, fMRI can reveal abnormalities in functional connectivity and microstructural changes in parallel (Babaeeghazvini et al.2021). Importantly, fMRI can also be used during transcranial magnetic stimulation (TMS) (Zhan & Yu, 2015) (Jonckers et al., 2015). Research has investigated how structural, functional, and connective properties of the motor cortex and other brain regions change after TMS to improve the understanding of TMS and, ultimately, its application in treating diseases such as ALS, which affect the NMJ (Bhattacharjee et al.2021)

Alterations in NMJ function can impact the form and frequency of neural firing in the motor neuron, affecting the excitability of the muscle fiber (Verma et al.2022)(Borzuola et al.2020). Recent findings that TMS can induce positive effects on muscles weakened by ALS, potentially enhancing NMJ function, may have interesting implications (Rawji et al.2020). The initial findings of NMJ disorders at the level of the motor neuron (excitability and firing patterns) and the muscle fiber (potentiation and modulation of NMJ transmission) raise interesting questions with important basic (understanding NMJ physiology) and clinical implications. To examine these questions in detail, fMRI will be used to explore how models of early-stage NMJ disorders, whether affecting the motor neuron (mature SOD1 model) or the muscle fiber (germline Agrin KO), affect the motor cortex and how possible compensatory mechanisms emerge to ameliorate functional deficits.

6. Neuroimaging Findings in Specific Neuromuscular Junction Disorders

Neuroimaging findings in myasthenia gravis. The possible neuroimaging manifestations of MG are discussed, emphasizing the role of magnetic resonance imaging (MRI) and computed tomography (CT) in diagnosing lesions of the thymus gland. Patients with MG may have detectable thymic pathology with hyperplastic thymus in about 30% and thymoma (thymic neoplasm) in about 10% of the cases (Pia Giannoccaro et al., 2021). MRI is the preferred non-invasive imaging modality to evaluate lesions of the thymus gland (Strange et al.2022). On T2-weighted MRI images, a hyperplastic thymus appears heterogeneous due to varying degrees of fatty replacement and may extend into the neck with an elongated shape (Wee et al., 2021). MRI is even capable of demonstrating abnormal thymic tissue after a successful thymectomy. The main types of thymoma are type B1 and B2 (WHO classification) associated with MG, and they appear mostly isointense or hypointense on T2-weighted images usually with cystic changes (Ohira et al.2022). Corticosteroid therapy may induce an increase in the size of thymoma.

Neuroimaging findings in Lambert-Eaton myasthenic syndrome. LEMS is an autoimmune presynaptic disorder of the NMJ mediated by autoantibodies directed against voltage-gated calcium channels (VGCCs) resulting

in reduced amount of acetylcholine release. It is classically recognized to be associated with malignant tumors, mostly small cell lung cancer (SCLC) (Fala et al., 2019). MRI of the thorax may show a mediastinal mass suggestive of neoplastic process such as a bronchial carcinoid tumor, teratoma, or lymphoma. Detection of the underlying malignancy is particularly important for optimal treatment and evaluation of prognosis.

6.1. Myasthenia Gravis

Myasthenia gravis is a prototype of a neuromuscular junction disorder characterized by weakness of striated muscles. Muscle fatigue is an important symptom of the disease. Since the ionic post-synaptic currents generating muscle activity and detecting electromyograms are small, it is difficult to access the critical muscle junction. Experimentally, animal models of Myasthenia gravis were created–using a variety of means–but successful diagnoses and therapeutic approaches for muscular underdevelopment are limited. Immunological disorders affect neuromuscular junction disorders that cause weakness of striated muscles. A best-known example is myasthenia gravis characterized by specific autoantibodies forming against nicotinic receptor subunit alpha (nAChR alpha). nAChR antibodies block transmission across the diaphragm-cleft post-synaptic membrane (Vilquin et al., 2019). For Myasthenia gravis, the cause of illness was uncovered more than 30 years ago, when it was demonstrated that the nicotinic acetylcholine receptor at the neuromuscular junction is attacked by the own antibodies of the patients, leading to endocytosis and internalization.

The imaging of the thymus in patients with myasthenia gravis is discussed from hyperplastic fatty replacement to thymic tumor. The imaging features of the normal thymus were also emphasized since they are well-established and quite different from those of the abnormal thymus. In addition, the potential technical improvements of THY-MRI and CT are presented (J. Strijkers et al., 2019). Overall, THY-MRI and CT are both viable methods for imaging the thymus with advantages and disadvantages specific to each modality. In myasthenia gravis patients, it is essential to perform thymus imaging to assess its structural changes before and after surgical intervention (Chowdhury & Chowdhury).

6.2. Lambert-Eaton Myasthenic Syndrome (LEMS)

This section details the neuromuscular junction (NMJ) disorder with imaging findings specific to Lambert-Eaton myasthenic syndrome (LEMS) (Erik Gilhus, 2011). The knowledge of imaging manifestation in LEMS is essential to understand the diagnostic utility of neuroimaging in neuromuscular junction disorders.

LEMS is a presynaptic NMJ disorder of motor fibers caused by antibodies against the voltage-gated calcium channels at the pre-synaptic motor terminals. It is often due to small cell lung carcinoma, leading patients to present with proximal limb weakness, ocular and bulbar symptoms, blepharoptosis, and lack of pupillary involvement (Jayarangaiah, Lui, & Kariyanna, 2023). Initial CT of the thorax might be done based on suspicion of lung mass found on history and examination. Sensitivity of MRI of the chest is less than 90%. Prior to treatment, LEMS patients had a characteristic, specific pattern of increased muscle uptake with preserved visualized post-synaptic activity best appreciated on R-RR prolonged and dynamic images (Fala et al., 2019).

7. Challenges and Limitations of Neuroimaging in Neuromuscular Junction Disorders

The combination of two or more neuroimaging modalities can have a great advantage in the study of NMD. A combination of μPET imaging and 7T-MRI has been proposed in dystrophy animal models, to analyse the relationship between atrophy, inflammation, and deterioration of NMJ (Zhang et al., 2020)The same combination approach will also be explored in TDP-43 pathological transgenic mice, to investigate TNF-α and IL-1β alteration in association with the degeneration of motoneurons and atrophy of anterior horn in spinal cord, NMJ and denervation of muscle fibres (J. Strijkers et al., 2019). There have also been calls to couple MRI to MEG, EEG or electrocardiogram (ECG) to localize lesions better or improve temporal resolution.

Despite all this effort and though neuroimaging has already contributed to research in the area of NMJ disorders, there are still several challenges and limitations to adopting it on a larger scale. Firstly, most techniques still suffer from interpretational challenges. NMJ disorders can be systemic as well as focal (Rodríguez Cruz, Cossins, Beeson, & Vincent, 2020). NMJ signal alteration can also represent other processes at play in the same system area, such as focal myopathy or myositis (Rodríguez Cruz et al., 2020) While there have been great efforts to identify possible neuroimaging markers specific to NMJ disease, such as the presence of specific immunoglobulin sub-classes in MG or autoimmune LEMS, more markers are still needed to better discriminate NMJ disorders from other diseases (Mejia Maza et al., 2021).

Another limitation of techniques is more technological. Standardization and multicentric application of techniques such as nm-joint MRI relies on the establishment of quality guidelines, to ensure that future experiments can be compared. It is foreseeable that, as in the field of the brain, certain neuromuscular structures are poorly

assessable at higher Tesla values. Therefore, in the face of progress in this area, it will be important to keep lower Tesla magnets in the game for specific investigation of certain structures.

7.1. Interpretation Challenges

Focusing specifically on the interpretation challenges, this subsection delves into the complexities and limitations associated with interpreting neuroimaging findings in the context of neuromuscular junction (NMJ) disorders. It offers a nuanced understanding of the obstacles and considerations relevant to neuroimaging interpretation.

The task of interpreting neuroimaging findings is often more complicated than that of acquiring the images (J. Strijkers et al., 2019). While modern sensors produce images with a high degree of repeatability, interpretation often relies on comparing findings with limited databases. When assessing muscle pathologies through a neuromuscular junction (NMJ) perspective, interpretation can become complex due to various factors (Iyer, Shah, & Lovering, 2021).

Static structures (the intra and extra cellular parts of NMJ) are difficult to standardize. There is an obvious variability in the shapes and types of synapses, the staining protocols used, and the machine settings (Davis, Fogarty, Brown, & Sieck, 2022). All of these factors influence the physical aspect of NMJ contours in images, thus making it difficult to compare anatomical data. Image acquisition and preparation even influence the physiological results derived from these structures (Mejia Maza et al., 2021). Several software have arisen to facilitate the automatic detection of static structures in images such as neuromuscular junctions, but interpretation is challenging on whether the parameters to be explored can be directly associated with a single physiology knowing that neuromuscular junctions (NMJs) can be intrinsically different to begin with (Vila, Qu, & Vunjak-Novakovic, 2020). In general, software is available to facilitate interpretation, but few know how to "calibrate" it to their own needs, easing false results. This bottleneck of expertise casts a long shadow on the advantageous development of meticulous models for a better understanding of physiology.

7.2. Technological Limitations

While great advances have been made in imaging techniques, current image technologies still impose technological limitations. Recent advances in neuroimaging approaches for characterizing NMJ disorders are still separately based on the democracy of the diseases and can complement each other (F. Vila et al., 2019). fMRI-based neuroimaging has recently demonstrated its feasibility for studying different NMJ disorders via percent change of the BOLD signal (task-related) and spontaneous BOLD signal fluctuation (task-free) (Stiernman et al., 2023). However, at present, no sensitive and specific imaging techniques are available to image the autonomous nervous system. Although MRI scanning can be standardized with related signal quantification metrics and possibly with ML approaches, the image quality is still typically heterogeneous across different scanners mainly due to differences in hardware component designs and calibration (Shimron & Perlman, 2023). The reliability of using these approaches for pre-clinical pharmaceutical studies may still need validation by combined histological analysis in an animal model and clinical trials in patients afterward. At present, scalp EEG can only provide information on slow NMJ activity, while high-frequency activity, which may be the main pathology for myotonic disorders, is beyond its detection. However, the low spatiotemporal resolution of current neuroimaging techniques remains a major challenge for characterizing different NMJ disorders. Recent advances in image reconstruction and signal separation methods, and widespread use of image analysis software has improved the availability of multi-dimensional raw data, and thus better explored the information in the form of both spatial and temporal domains in one study (J. Strijkers et al., 2019). It is believed that development in this direction may result in improvements to the spatiotemporal resolution of current imaging techniques and significant advances in studying different aspects of NMJ processes.

8. Future Directions in Neuroimaging Research

Neuroimaging holds great promise for the future study of the neuromuscular junction (NMJ) and its disorders. Using recent advances in ultrafast imaging, genetically encoded or optophysiological biosensors, multiplexed imaging of neurons and the NMJ is now possible (Liu et al., 2015). Further studies of the NMJ in defined disease states will allow for a better understanding of the earliest events in synaptopathies. Additional biosensors for synaptic activity and muscle action potentials will enable parsing the contribution of pre- versus postsynaptic dysfunction in NMJ disorders and provide a framework for neuronal rewiring and compensatory responses. Electron and high-resolution light microscopy can be used concurrently with fluorescent imaging, revealing the spatial arrangement of neuromodulatory and signaling molecules at the NMJ and their potential roles in synaptic plasticity and restoration of function (Barrantes, 2022).

Compressive-sensing techniques combined with microendoscopic imaging provide one avenue to achieve high-resolution imaging of deeper structures across a large field of view (McMackin et al., 2019). New lenses with

extreme fields of view, and atomically flattened gold surfaces for high-resolution silver-enhanced enhancement of gold nanoparticles with multi-color imaging covalently attached to multiple targets, allow imaging across several different scales and tissue types (Mieszawska, Mulder, Fayad, & Cormode, 2013). Integrated platforms incorporating microfluidics, microelectrodes, and drug delivery will enable multimodal (electrophysiology, imaging, and pharmacology) interrogation of the role of signaling pathways in synaptic maintenance. The development and application of genetically encoded biosensors for optical imaging of neuroactivity at the individual cellular level will enable further insight into the dynamic actions of epigenetic regulators, transcription factors, and growth factors in synaptic development and stabilization.

8.1. Emerging Technologies

Focusing on the emerging technologies within, including those with only recent- or currently-proposed applications. Understanding emerging technologies, especially newly conceived ideas or those that are still under development, will help to direct thinking about where neuroimaging might be heading in a few years, and what kinds of issues may arise from particular technologies in future use. Where relevant, topics include expanding beyond the focus on muscles of the limbs to cover those of the eyes or diaphragm. Also, consider using complementary techniques concurrently or sequentially in the same patient to best leverage the advantages and address the limitations of each. These emerging MRI technologies have potential relevance to understanding and/or treating disease at the NMJ. Fine needle electromyography is a proposed technology that may enhance the diagnosis of certain NMJ disorders (Patel & Pobre, 2020). 12-lead electromyography is a proposed technology that may enhance the diagnosis of certain NMJ disorders. Optical stimulation of nerve and EMG recording is a technology just beginning to be tested in animals which holds potential promise for enhancing the understanding of NMJ disorders (Patel & Pobre, 2020). Chip-based technologies for monitoring cell-cell interactions, Ca waves, and temperature changes in the vicinity of single mitochondria are technologies that are primarily relevant to drug discovery and perturbation of cell signaling but could have potential applications in neuroscience understanding NMJ function (J. Strijkers et al., 2019).

8.2. Potential Applications in Clinical Practice

Advances in neuroimaging are now extending their potential application into clinical practice. Insufficient neuroimaging has severely hindered the systematic clinical study into any aspect of the NMJ (neuromuscular junction) (H. Paul, 2016). Due to recent dramatic improvements in high-resolution structural imaging techniques, new avenues of research have opened for the study of NMJ disorders. These potential implications of neuroimaging technology developments for the clinical diagnosis and understanding of NMJ disorders are discussed.

As imaging technologies continue to evolve, insights into cellular-level mechanisms of NMJ disruption in early pathogenesis can potentially be gained from both ballistic and structural imaging, which may complement broader signal-based imaging (McMackin et al., 2019). In AD (Alzheimer's disease) mouse models, for example, metabolic changes, neurovascular uncoupling, and altered neurophysiological signaling, such as increased gamma-frequency oscillations, have been observed prior to morphological alterations (Ramlakhan et al., 2020). In this disease model, high-resolution imaging appears to treat two aspects of NMJ function (maintenance and disruption) differently. Though neuroimaging can visualize responses to structural neurochef disruption in virtually all NMJ disorders, understanding the etiological events leading to dysfunction is difficult.

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