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Review

Electrophysiological analysis of neuromuscular synaptic function in myasthenia gravis patients and animal models

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ABSTRACT

Study of the electrophysiological function of the neuromuscular junction (NMJ) is instrumental in the understanding of the symptoms and pathophysiology of myasthenia gravis (MG), an autoimmune disorder characterized by fluctuating and fatigable muscle weakness. Most patients have autoantibodies to the acetylcholine receptor at the NMJ. However, in recent years autoantibodies to other crucial postsynaptic membrane proteins have been found in previously 'seronegative' MG patients. Electromyographical recording of compound and single-fibre muscle action potentials provides a crucial in vivo method to determine neuromuscular transmission failure while ex vivo (miniature) endplate potential recordings can reveal the precise synaptic impairment. Here we will review these electrophysiological methods used to assess NMJ function and discuss their application and typical results found in the diagnostic and experimental study of patients and animal models of the several forms of MG.

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Introduction

The neuromuscular junction (NMJ) is perhaps the most-studied and best-understood synapse of the nervous system. For many decades it has served as a model synapse, enabling the discovery of universal synaptic principles. Electrophysiological study of synaptic signalling has provided critical insights into the mechanisms of neurotransmission. Here, we will review the electrophysiological methods widely used to measure NMJ function in vivo and ex vivo and discuss their application in the study of the NMJ disorder myasthenia gravis (MG) and its animal models.

MG is hallmarked by fluctuating and fatigable muscle weakness. Most patients (~85%) have serum autoantibodies against the acetylcholine (ACh) receptors (AChRs) on the postsynaptic membrane at the NMJ. In recent years it has become apparent that a large proportion (40–70%) of the AChR-negative MG patients are seropositive for antibodies against muscle-specific kinase (MuSK), a postsynaptic membrane tyrosine kinase that forms the core of a multi-protein signalling complex. MuSK is vitally involved in the embryonic development, and subsequent maintenance of AChR clusters at NMJs (Hoch et al., 2001; Koneczny et al., 2014). Some of the remaining MG patients (seronegative for both AChR and MuSK antibodies) instead have antibodies against low-density lipoprotein receptor-related protein 4 (LRP4) (Higuchi et al., 2011; Pevzner et al., 2012; Zhang et al., 2012). LRP4 is a membrane receptor that gets activated by neurally released agrin and stimulates MuSK activation to drive AChR clustering (Ghazanfari et al., 2011). Very recently anti-agrin antibodies have also been detected in some MG sera, mostly in those patients who are also seropositive for either AChR, MuSK or LRP4 antibodies (Gasperi et al., 2014; Zhang et al., 2014). In addition, some previously AChR 'seronegative' MG cases appear to have low-affinity antibodies that can be detected in a sensitive cell-based assay in which AChRs are clustered on the cell surface (Leite et al., 2008).

AChR MG can be classified into an early-onset group (age <40 years) with female predominance (often with thymic hyperplasia), and a late-onset group in which males predominate and with mostly a normal (i.e. atrophied) thymus (Sieb, 2014; Verschuuren et al., 2013). Thymoma is present in ~10% of AChR MG patients with generalized weakness. It is seen across all ages, although somewhat more often in the elderly. About 20% of AChR MG patients display weakness of only their extraocular muscles. MuSK MG has distinct features. It occurs at higher frequency in women, with the peak incidence in the 4th age decade as compared to the 3rd decade for AChR MG. Often there is prominent oropharyngeal, facial, neck and respiratory muscle weakness and facial and

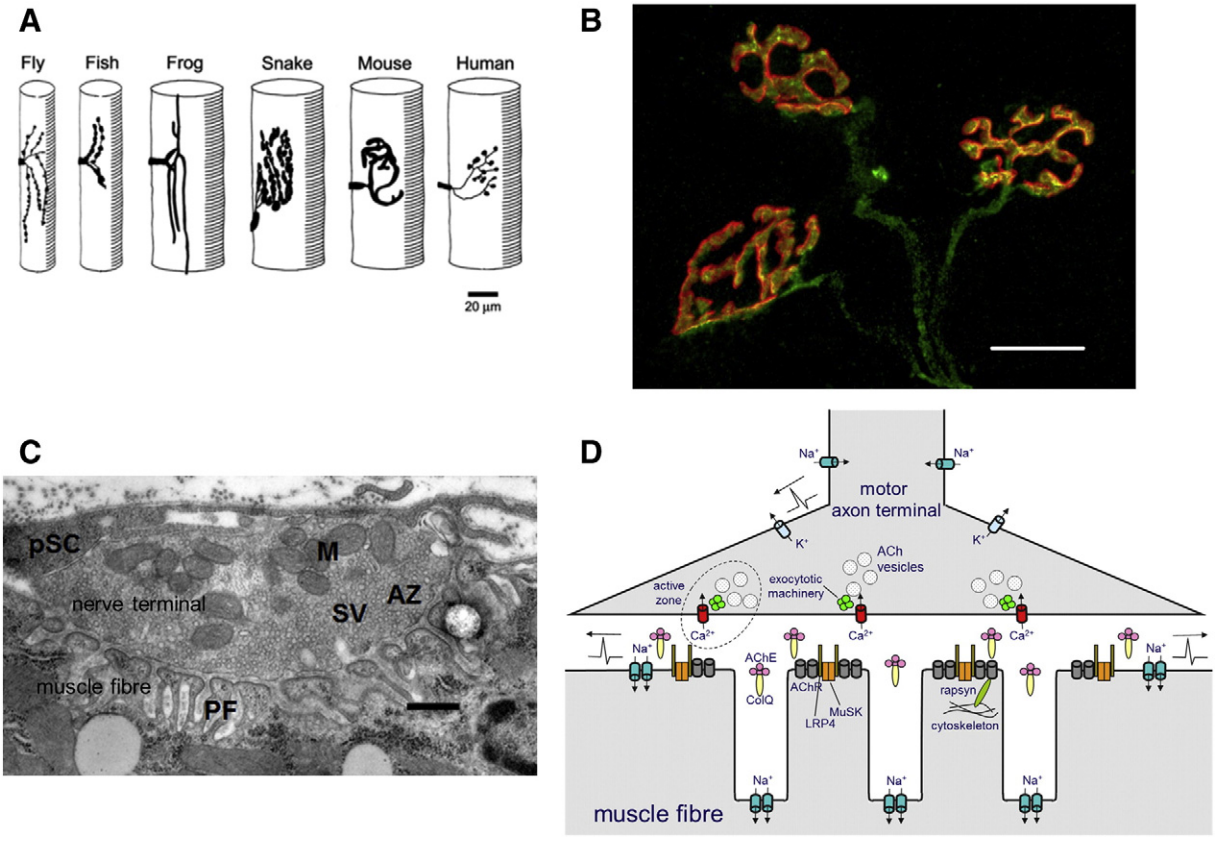

Fig. 1. Structure of the neuromuscular junction. (A) Inter-species morphological variation of the neuromuscular junction (NMJ). Axon terminal processes are shown in black on the muscle fibre surface. Taken from (Slater, 2008), with permission. (B) En face image of three pretzel-shaped mouse diaphragm NMJs (confocal microscopy z-projection). Acetylcholine receptors (red) are stained with Alexa Fluor 555-α-bungarotoxin and nerve terminals (green) with anti-synaptophysin antibody (scale bar = 20 µm). (C) Electron microscopical picture of a nerve terminal profile. pSC = perisynaptic Schwann cell; M = mitochondria; SV = synaptic vesicles; AZ = active zone; PF = postsynaptic folds (scale bar = 0.5 µm). (D) Schematic drawing of key proteins involved in neuromuscular transmission (ACh = acetylcholine; AChE = acetylcholinesterase; ColQ = collagen-Q; LRP4 = low-density lipoprotein receptor-related protein 4).