Current concepts in neuromuscular transmission

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The neuromuscular junction (NMJ) is structured and powered to transduce electrical activity from the distal nerve terminal of a motor neurone via the neuromuscular cleft to the post-junctional muscle membrane to ultimately generate muscle contraction. Our understanding of this complex function has expanded over many years, and the NMJ has served as a prototype for how different synapses operate in the peripheral and central nervous systems. The NMJ has a presynaptic part which is synonymous with the distal nerve ending, being responsible for neurotransmitter synthesis, packaging into vesicles, and subsequent vesicle transportation to active release sites where vesicle docking, fusion, and release of acetylcholine and other co-released transmitters finally take place. The synaptic cleft, filled with large molecular complexes that guarantee ultrastructural NMJ arrangement and signal transduction, allows for rapid diffusion and degradation of the neurotransmitter. The postsynaptic part consists of a folded muscle membrane into which nicotinic acetylcholine receptors (nAChRs) directly opposite the presynaptic active release sites are mounted and fixed by a cytoskeleton. This specialized postsynaptic region is closely associated with the perijunctional zone where a high density of sodium channels promote and amplify the signal in order to guarantee the propagation of the electrical activity to generate muscle contraction. The transduction process is maintained at load (i.e. high stimulus frequency) by a presynaptic mechanism allowing for sustained transmitter release over time at high demand. This positive feedback mechanism relies on neuronal nAChRs present on the distal nerve terminal, whereas the continuation of the transduction process at the postsynaptic part relies on the classical muscle type nAChR.

In this review, we will focus on recent findings of potential clinical importance that will advance our understanding of the effects of neuromuscular blocking agents and neuromuscular monitoring and also our management of disorders of the neuromuscular system within anaesthesia and intensive care.


Keywords: acetylcholine; neuromuscular block; neuromuscular transmission, synapse; receptors, cholinergic; receptors, postsynaptic; receptors, presynaptic

The neuromuscular junction (NMJ) is one of the most widely studied synapses and has served as a prototype for our understanding of the transmission of electrical activity and subsequent communication between neurones and effector cells within the central and peripheral nervous systems. The phenomenon of neuronal cross-talk is often termed neurotransmission. Electrical neurotransmission and the presence of chemical compounds with a critical function for the transmission of information from nerve to muscle was first described by Claude Bernard in his pivotal series of studies of the effects of curare on nerve-muscle preparations. In contrast to what later became evident, Bernard overlooked the possibility of a specific junction between nerve and muscle and suggested that curare blocked muscle contractions primarily by a direct action on the nerve. Vulpian later proposed that there must be a distinct junction between the nerve and the muscle and his suggestions were confirmed and further advanced by Langley who in 1905 demonstrated the role for a chemical compound released by the nerve and transmitted across the NMJ onto the muscle to initiate a muscle contraction.

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In this review, we will focus on recent findings concerning neuromuscular transmission that we believe are of clinical importance and that will advance our understanding of the effects of neuromuscular blocking agents (NMBAs) and neuromuscular monitoring and also our management of disorders of the neuromuscular system within anaesthesia and intensive care.

The neuromuscular junction

Presynaptic part, synaptic cleft, and postsynaptic part

The NMJ is responsible for chemical transmission of electrical impulses from nerve to muscle in order to generate an appropriate muscle contraction. To fulfil this task, the junction has a complex cellular and molecular architecture that involves specialized regions to allow sustained release of neurotransmitters at various loads, allowing for safe neurotransmission. The junction consists of three distinct parts: the distal motor nerve ending, also termed the presynaptic part; the synaptic cleft; and the postsynaptic part which is a part of the muscle membrane. These elements together form the NMJ where the information from nerve to muscle is transmitted via the instantaneous release of acetylcholine (ACh) and activation of ligand-gated, fast-acting nicotinic acetylcholine receptors (nAChRs).

The presynaptic part consists of the distal part of the motor neurone, that is, the distal and demyelinated part of the motor nerve axon. This myelin-free part of the nerve axon is more or less encapsulated by a terminal Schwann cell surrounding the anchoring of the nerve ending into the muscle membrane. The role of the Schwann cell is primarily to support the connection between nerve and muscle and to promote survival of motor neurones. This function is achieved by the release of nerve growth factor and neuregulin from the Schwann cell and by reactions to trophic factors released by the distal nerve terminal. The Schwann cells are not primarily involved in chemical transmission, but have key roles in the maintenance of the nerve terminal: they may also promote nerve terminal regeneration after damage to the terminal. In this context, the presynaptic Schwann cell may take a phagocytic role and by removal of damaged cell material clean the presynaptic area and prepare for nerve terminal regeneration in order to promote renewed motor neurone attachment to the postsynaptic muscle membrane.

The nerve terminal is responsible for the release of neurotransmitter over the synaptic cleft and it also has cholinergic autoreceptors, which when activated by ACh stimulate release of more ACh from the presynaptic terminal. The terminal is also involved in the maintenance of appropriate junctional architecture. Thus, this presynaptic part of the NMJ is designed and powered to guarantee neurotransmitter synthesis and transmitter incorporation into vesicles, neurotransmitter release, and reuptake, and for transportation of ions across the nerve terminal cell membrane. The energy required for these processes is generated by a large population of mitochondria present in the cytoplasm and typically visible during electron microscopy of the NMJ.

ACh is the primary neurotransmitter that is synthesized, stored, and released by the nerve terminal. The actual mechanisms by which ACh is packed and stored in nerve terminal vesicles are still not fully understood. In short, ACh is synthesized from acetate and choline and packed into vesicles by an active energy-dependent process. Vesicles are stored after packaging in two major sites: one is located at an active site near the presynaptic cell membrane and immediately opposite the ACh receptor on the postsynaptic muscle membrane, thus the ACh is readily releasable and with the shortest distance to travel to the postsynaptic site. Although the general opinion has been that vesicles closest to the cell membrane are preferentially released upon the arrival of an action potential, other studies suggest that vesicles ready to be released are randomly distributed within this membrane close cluster. Other vesicles are stored at a more remote position and have to be transported via the cytoskeleton down to the active sites for release.

The subsequent docking, fusion, and release of neurotransmitter (exocytosis) is a complex mechanism; the detail of which is not the focus of this review (see also Sudhof). In brief, when an action potential arrives at the distal nerve terminal, voltage-gated calcium channels, located here at high density, open to generate a local inward calcium flux leading to increased intracellular calcium concentration. At this stage, three proteins within the group of soluble N-ethylmaleimide-sensitive-factor attachment receptor (SNARE) proteins, synaptobrevin, syntaxin, and synaptosome-associated protein SNAP-25, have key functions in the attachment of vesicles to the cell membrane. The locally increased calcium concentration is sensed by specific vesicle membrane-associated synaptotagmins. Activated synaptotagmins mediate the formation of complexes of the three SNARE proteins that ultimately leads to vesicle docking and fusion and subsequent release of neurotransmitter into the synaptic cleft. Used vesicle and membrane parts are recycled through active transportation into the nerve terminal where they are reused to form new vesicles, packed with neurotransmitter, and then transported to the active sites via the cytoskeletal transport system ready for release.

Under resting conditions (absence of a nerve action potential at the distal nerve terminal), small spontaneous endplate potentials can be recorded at the postsynaptic side of the NMJ. These miniature endplate potentials (MEPP) are the result of release of a small number of ACh-containing vesicles, possibly even a single vesicle and are often termed quantal release. When an action potential arrives at the nerve terminal, several hundred vesicles are released synchronously resulting in a summation of
corresponding MEPPs to give an endplate potential. If the amplitude of this endplate potential is large enough to depolarize the muscle cell, it triggers the propagation of the action potential along the postsynaptic membrane, ultimately leading to calcium release within the muscle and the actin–myosin interaction that results in muscle contraction.4

The synaptic cleft spans ~50 nm from the nerve ending to the muscle membrane. The cleft contains a basal lamina made up of a multitude of large molecules forming an extracellular matrix that aids cell adhesion and appropriate neuromuscular signalling processes. Here, molecules such as acetylcholinesterase, various isoforms of the alpha- and beta-laminin, agrin, and collagen interact and modulate conditions for appropriate neurotransmission. Locally, high concentrations of neurotransmitter in combination with the short distance from the active sites of release to the opposite postsynaptic membrane promote rapid diffusion of the transmitter. ACh is degraded by acetylcholinesterase in the synaptic cleft. Here, acetylcholinesterase is anchored to the lamina from where it rapidly degrades the transmitter by splitting ACh into acetate and choline. This degradation of ACh in the cleft is responsible for the termination of its action. In addition, an antagonist of acetylcholinesterase will have to reach the synaptic cleft before it can antagonize the enzyme and thereby prolong the action of ACh.

The postsynaptic membrane consists of multiple folds located opposite the presynaptic nerve terminal. These primary (shallower) and secondary (deeper) folds of the postsynaptic membrane expand its surface area many-fold. On the shoulder of these folds, nAChRs cluster at high density and are anchored into the cell membrane by a complex system of cytoskeletal proteins, for example, dystroglycans.13 Neurotransmitter release has been shown to play a role in the structure of the NMJ and more specifically the ultrastructure of the postsynaptic membrane. Interestingly, adenosine triphosphate co-released with ACh and involved in P2X2-receptor-mediated signalling, was recently shown to have a key role in the formation of postsynaptic structures.30

In close proximity to the highly specialized postsynaptic membrane is the so-called perijunctional zone. This part of the NMJ has a crucial function in the transduction of the signal from the junction and further into the muscle cell. Typically, the perijunctional zone has a higher density of sodium channels than in other parts of the cell membrane, making this part of the muscle membrane more capable of amplifying the responses to depolarization and thus to promote the transduction process that finally leads to muscle contraction.4

Nicotinic ACh receptors
The most important receptors in the neuromuscular junction are the nicotinic acetylcholine receptors (nAChRs). Present both pre- and postsynaptically, the nAChRs mediate fast neurotransmission by the action of ACh. Being the most studied receptor, the nAChR is the prototype for the cys-loop superfamily of ligand-gated ion channels which also includes GABA\textsubscript{A}, glycine and 5-HT\textsubscript{3} receptors. All members of the cys-loop ligand-gated ion channel superfamily share a common structure and function. They have a common architecture with five subunits surrounding a central pore and each subunit is built upon four transmembrane segments, where the second transmembrane segment lines the pore. Activation of the nAChR by ACh leads to an influx of cations (i.e. Na\textsuperscript{+} and Ca\textsuperscript{2+}) that lead to depolarization of the cell membrane.

The nAChRs have been subdivided into muscle and neuronal subtypes based on their classical major site of expression; however, recent data indicate that many neuronal nAChRs are expressed in non-neuronal tissue (see below). At present, 17 nicotinic subunits have been cloned, the muscle \(\alpha_1, \beta_1, \delta, \gamma\) and \(\epsilon\) subunits, and the neuronal \(\alpha_2-\alpha_{10}\) and \(\beta_2-\beta_4\) subunits. The \(\alpha\) subunits have two adjacent cysteines essential for ACh binding, but both the \(\alpha\) subunit and the non-\(\alpha\) subunits contributes to the specificity within each receptor subtype.22 25 The subunits co-assemble to muscular \(\alpha_1\beta_1\gamma\delta\) and \(\alpha_1\beta_1\epsilon\delta\) subtypes and heteromeric and homomeric neuronal nAChRs. The muscle nAChRs will be discussed in detail below (Postsynaptic nAChRs). The neuronal nAChRs include both homomeric and heteromeric receptors, with the \(\alpha_7-9\) subunits forming homomeric nAChRs. The heteromeric receptors are formed by a combination of \(\alpha_2-6\) and \(\beta_2-4\), most commonly by a single \(\alpha\) and a single \(\beta\) subunit, with a stoichiometry of \(2\alpha\) and \(3\beta\). Although there are many potential combinations of neuronal nAChRs, only a few have been found to be of biological importance. Neuronal nAChRs are widespread in the human body. In the brain, the neuronal \(\alpha_4\beta_2\) nAChR is the major nicotinic receptor responsible for addiction to nicotine, whereas \(\alpha_3\beta_4, \alpha_3\beta_2\) and \(\alpha_7\) nAChRs are abundant in autonomic ganglia and in the adrenal medulla. Apart from being involved in multiple functions in the central nervous system, such as behaviour, cognitive function and memory, it is obvious that neuronal subtype nAChRs also play an important role in the modulation and regulation of several vital control systems in the peripheral nervous system, such as regulation of breathing, inflammation and in the immune system.

The neuronal nAChR subunits \(\alpha_3-\alpha_5, \alpha_7, \beta_2, \beta_4\) have been found to be of importance in oxygen signalling from oxygen sensing chemoreceptor type-1 cells in carotid body.31 Here, the neuronal nAChRs are critical parts in chemoreceptor signal transduction during hypoxia that, via the afferent carotid sinus nerve, transfer the information to central respiratory circuits of the brain stem to generate a hypoxic ventilatory response. Interestingly, the neuronal \(\alpha_7\) nAChR has been localized in macrophages and is found to be critical for the cholinergic-antiinflammatory
pathway which is a part of the inflammatory reflex. Briefly, cytokines activated by pathogens and ischaemia stimulates the afferent vagus nerve that via the dorsal motor nuclei activates a muscarinic (M1) agonist-responsive cholinergic brain network. Efferent signals from the vagus nerve inhibit cytokine release via activation of α7 nAChRs present on macrophages (for review see Tracey).

**Presynaptic nAChRs**

Nicotinic autoreceptors are localized at the presynaptic nerve terminal and are responsible for the increased release of ACh into the synaptic cleft during high frequency stimulation of the presynaptic nerve terminal. This cholinergic receptor is not inhibited by α-bungarotoxin, but rather by non-depolarizing NMBAs and hexamethonium, and has now been identified as the neuronal nAChR α3β2. Targeted pharmacological inhibition of the presynaptic α3β2 nAChR during high frequency repetitive (i.e. tetanic) stimulation causes a typical tetanic fade phenomenon. This tetanic fade seen during experimental conditions equates to the classical train-of-four (TOF) fade which is seen during clinical non-depolarizing neuromuscular block during neuromuscular monitoring. It was recently demonstrated that non-depolarizing NMBAs in clinical use, in contrast to the depolarizing NMBA succinylcholine, inhibit this α3β2 nAChR, and this explains why TOF fade is absent during depolarizing block.

**Postsynaptic nAChRs**

ACh released from the motor nerve ending binds to nAChRs at the postsynaptic membrane and causes depolarization of the membrane and ultimately muscle contraction. There are two types of muscle nAChRs, the fetal α1β1γδ and the adult α1β1εδ. These receptors co-assemble as 2:1:1:1 and require two ACh molecules for activation. There is a high and a low affinity binding site at the α-δ and α-γ/ε interface, respectively. In fetal life the muscle α1β1γδ nAChR is expressed. After birth, however, increased depolarization leads to dramatic increase in transcription of the ε subunit and the ε subunit drive out by competition the γ subunit for assembly into the receptor. The adult muscle α1β1εδ nAChRs preferentially aggregate at the neuromuscular junction, are more stable to degradation, and have a more rapid response to agonist compared to the α1β1γδ receptor.

During normal adult life, the α1β1εδ nAChR is expressed at the muscle membrane. However, during immobilization, denervation, or inflammation, the γ subunit remaining in the muscle nucleus starts to re-express at the muscle membrane. In addition, animal studies have demonstrated that the neuronal α7 nAChR subtype is also found in the muscle membrane during development and denervation, indicating that this receptor subtype might be associated with endplate stabilization and synaptogenesis.

In summary, three different subtypes of nAChRs are present at the postsynaptic muscle membrane, the muscle fetal α1β1γδ and adult α1β1εδ receptors and the neuronal α7 subtype.

**Clinical perspectives**

**Actions of NMBAs**

The two different classes of clinically used NMBAs prevent neuromuscular transmission by separate mechanisms. Non-depolarizing NMBAs, such as atracurium and rocuronium, have classically been described as competitive inhibitors of muscle nAChRs. This means that non-depolarizing NMBAs compete with the natural ligand ACh at the two binding sites of the nAChR. The clinical application of this is reflected by the use of anticholinesterase to prevent the degradation of ACh and thereby

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Fig 1 Structure of the α3β2 nAChR. (a) Lateral view of the receptor and (b) schematic presentation of the acetylcholine binding sites.
increasing the concentration of ACh favouring neuromuscular transmission. Notably, only one molecule of non-depolarizing NMBA is needed in order to prevent activation of the receptor whereas two molecules of ACh are necessary for activation. Thus, the inhibition by NMBA is preferable. Interestingly, recent findings indicate that the mechanism of inhibition of muscle nAChRs by non-depolarizing NMBA is more complex than just competitive inhibition. The clinical consequences of this finding are unclear and more studies are needed. In the clinical setting, a non-depolarizing neuromuscular block displays a typical TOF fade and a reduction in twitch amplitude directly reflecting the action of the NMBA at different subtypes of nAChR. Inhibition of the presynaptic α3β2 nicotinic autoreceptor by non-depolarizing NMBA creates the TOF fade, whereas the reduction in twitch amplitude is the result of inhibition of the muscle nAChR at the postsynaptic muscle membrane (Fig. 2). Hence, neuromuscular monitoring is so far the only way by which we directly measure the effect of a drug (i.e. non-depolarizing NMBA) at target receptors. Moreover, a depolarizing NMBA (i.e. succinylcholine) reduces the twitch amplitude but lacks TOF fade, which fits well with its distinct action at the muscle nAChR but has low affinity to the presynaptic α3β2 nAChR. However, despite more than 50 years of clinical use, the exact mechanism of action of succinylcholine is still not clear. While the initial fasciculations seen after administration of succinylcholine reflects the massive activation of muscle nAChRs at the postsynaptic muscle membrane, the subsequent paralysis probably reflects desensitization of the muscle receptor, inactivation of voltage-gated sodium channels and increase in potassium permeability in the surrounding membranes.

**Diseases**

Several medical conditions interfere with neurotransmission at the NMJ and the clinical use of NMBA.

Botulism is a fatal disease caused by botulinum toxin and is characterized by a flaccid paralysis caused by proteolytic cleavage of SNARE proteins critically involved in the release of ACh from nerve endings. Toxin-induced inhibition of ACh release causes disturbance in all synapses releasing ACh. The clinical features of botulism include muscle paralysis leading to respiratory failure but may also involve cholinergic signalling in the heart with a subsequent risk for cardiovascular collapse.

Other disorders affecting the presynaptic site in the NMJ are the Lambert–Eaton syndrome and neuromyotonia. The Lambert–Eaton syndrome is an autoimmune disorder with autoantibodies against P/Q-type voltage-gated calcium channels presynaptically in both the NMJ and the autonomic nervous system, thus resulting in muscle weakness, fatigue and autonomic dysfunction. In contrast, autoantibodies against presynaptic potassium channels in neuromyotonia causes increased presynaptic transmitter release and the hyperexcitability. These patients can have severe muscle cramps, stiffness, and myokymia (muscle twitching during rest).

Myasthenia gravis is the most common disorder affecting the postsynaptic part of the NMJ. It is caused by autoantibodies against muscle nAChRs leading to muscle weakness. Increased presynaptic release of ACh may partially compensate for the reduction in functional muscle nAChRs at the postsynaptic membrane. However, these patients are often treated with anticholinesterases in order to increase the levels of ACh and thereby normalize the synapse.

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*Fig 2* The effect of NMBA on pre- and postsynaptic nAChRs serve as the molecular basis for the TOF phenomenon. Denervation cause expression of fetal muscle type nAChR and the neuronal type α7 nAChR.
NMBAs also have the potential to cause pathological states. There is increased evidence that succinylcholine can cause severe hyperkalaemia, even leading to malignant arrhythmias and cardiac arrest, if used in patients with prolonged immobilization, inflammation or denervation.\(^2\) The mechanism behind this complication is believed to be a combination of upregulation of both fetal and adult muscle nAChRs and extrajunctional expression of fetal muscle nAChRs induced by immobilization, denervation or inflammation.\(^2\) In addition, succinylcholine has a higher affinity for the fetal muscle nAChR, thus the activation of the fetal receptor is easier and when activated, the receptor stays open for a longer time, consequently producing a greater response.\(^2\)

During recovery from a neuromuscular block, residual effects will primarily target vital control of breathing and of airway integrity. Non-depolarizing NMBAs reduce not only the acute hypoxic ventilatory response (HVR) due to an interaction with carotid body chemosensation\(^6\)\(^7\) but, also, pharyngeal function and airway control\(^8\)\(^9\) in normal volunteers. Depressed HVR and increased risk for aspiration and airway impairment are consequently seen at partial paralysis corresponding to a mechanical adductor pollicis TOF ratio (T4/T1) of \(<0.90\). This has led to the recommendation that the TOF ratio should be \(>90\)% before extubation, to allow adequate respiratory control and airway integrity.\(^37\) Recently, the attenuation of hypoxic ventilatory response by non-depolarizing NMBAs has been traced to an inhibition of neuronal nAChRs in the carotid body.\(^3\)\(^7\)\(^15\)\(^18\)

Finally, involvement of the \(\alpha_7\) nAChR in the cholinergic anti-inflammatory pathway may in the future have clinical implications concerning the use of non-depolarizing NMBAs in patients with sepsis, haemorrhagic shock, and ischaemia-reperfusion injury in the intensive care unit, since it is demonstrated that non-depolarizing NMBAs inhibit the \(\alpha_7\) nAChR.\(^3\)\(^7\)\(^16\)\(^18\)\(^36\)

In summary, our understanding of the complex structure and action of the NMJ have expanded over the years due to molecular biology and imaging techniques. Current concepts in receptor physiology and pharmacology have increased our knowledge about the precise action of NMBAs. Also, it serves as a basis for the principles of neuromuscular monitoring as well as for how NMBAs interact with other vital regulatory systems in respiration, inflammation, and in the immune system.

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