Neuromuscular Transmission
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(Dr. DeSimone’s lecture notes revised)

Learning Objectives:

1. Know the subunit composition of nicotinic ACh channels, general topology of the \( \alpha \) subunits and basic i/v characteristics.
2. Sketch the important structures in a motor end plate giving the relative structures of the presynaptic terminal axon and the postsynaptic sarcolemma including: the presynaptic active zones, synaptic cleft, and postsynaptic junctional folds.
3. State the reason for the 1 msec delay in the time between the arrival of an action potential at the terminal presynaptic region and the first postsynaptic electrical activity.
4. State the function of ACh in the causal chain leading to the end plate potential (EPP).
5. State the mechanism by which the use of curare permits the separation of the EPP from the postsynaptic action potential and name the active agent in curare.
6. State the effect of ACh binding to the ACh receptor on the Na\(^+\) and K\(^+\) permeability of the ACh receptor.
7. State the most important mode of ACh removal from the synaptic cleft.
8. State the effect acetylcholine esterase inhibitors on the amplitude and duration of the EPP.
9. State the experimental results that indicate that the ACh receptor is a ligand-gated nonselective cation channel.
10. State the effect of depolarization on the Ca\(^{2+}\) permeability of the terminal presynaptic region.
11. State the effect of Ca\(^{2+}\) removal from the end plate region on the EPP.
12. Describe the experiment that proved the effect of Ca\(^{2+}\) during neuromuscular transmission is on the presynaptic side of the end plate.
13. Discuss the significance of MEPPs for ACh release.
14. Know the general activation mechanism for NMDA and non-NMDA channels and role in the physiology of central neurons (e.g. LTP).

Suggested Reading:

- See Lecture Notes and
- Physiology, 3rd Edition, L.S. Costanzo, Saunders, pp 24-28
**Neurotransmitter Receptors**

The diversity of neurotransmitters is extensive, but their receptors can be grouped into two broad classes: ligand-gated ion channels and G protein-coupled receptors. By far the most-studied receptor is the muscle nicotinic acetylcholine receptor, the first ligand-gated ion channel to be purified, cloned, and characterized at the molecular level. The structure and mechanism of this receptor are understood in considerable detail, and it provides a paradigm for other neurotransmitter-gated ion channels. When activated, these receptors induce rapid changes, within a few milliseconds, in the permeability and potential of the postsynaptic membrane. In contrast, the postsynaptic responses triggered by activation of G protein-coupled receptors occur much more slowly, over seconds or minutes, because these receptors regulate opening and closing of ion channels indirectly.

**Opening of Acetylcholine-Gated Cation Channels Leads to Muscle Contraction**

The nicotinic acetylcholine receptor, a ligand-gated cation channel, admits both K\(^+\) and Na\(^+\). Although found in some neurons, this receptor is best known for its role in synapses between motor neurons and skeletal muscle cells. Patch-clamping studies on isolated outside-out patches of muscle plasma membranes have shown that acetylcholine causes opening of a cation channel in the receptor capable of transmitting 15,000-30,000 Na\(^+\) or K\(^+\) ions a millisecond.

Since the resting potential of the muscle plasma membrane is near E\(_{k}\), the potassium equilibrium potential, opening of acetylcholine receptor channels causes little increase in the efflux of K\(^+\) ions; Na\(^+\) ions, on the other hand, flow into the muscle cell. The simultaneous increase in permeability to Na\(^+\) and K\(^+\) ions produces a net depolarization to about −15mV from the muscle resting potential of −85 to −90 mV. This depolarization of the muscle membrane generates an action potential, which – like an action potential in a neuron – is conducted along the membrane surface via voltage-gated Na\(^+\) channels. When the membrane depolarization reaches a specialized region, it triggers Ca\(^{2+}\) movement from its intracellular store, the sarcoplasmic reticulum, into the cytosol; the resultant rise in cytosolic Ca\(^{2+}\) induces muscle contraction.

Two factors greatly assisted in the characterization of the nicotinic acetylcholine receptor. First, this receptor can be rather easily purified from the electric organs of electric eels and electric rays; these organs are derived from stacks of muscle cells (minus the contractile proteins) and thus are richly endowed with this receptor. (In contrast, this receptor constitutes a minute fraction of the total membrane protein in most nerve and muscle tissues). Second, α-bungarotoxin, a neurotoxin present in snake venom, binds specifically and irreversibly to nicotinic acetylcholine receptors. This toxin can be used in purifying the receptor by affinity chromatography and in localizing it. For example, in autoradiographs of muscle-cell sections exposed to radioactive α-bungarotoxin, the toxin is localized in the plasma membrane of postsynaptic striated muscle cells immediately adjacent to the terminals of presynaptic neurons.
Careful monitoring of the membrane potential of the muscle membrane at a synapse with a cholinergic motor neuron has demonstrated spontaneous, intermittent, and random ~2-ms depolarizations for about 0.5-1.0 mV in the absence of stimulation of the motor neuron. Each of these depolarizations is caused by the spontaneous release of acetylcholine from a single synaptic vesicle. Indeed, demonstration of such spontaneous small depolarizations led to the notion of the quantal release of acetylcholine (see below) and thereby led to the hypothesis of vesicle exocytosis at synapses. The release of one acetylcholine-containing synaptic vesicle results in the opening of about 3000 ion channels in the postsynaptic membrane, far short of the number need to reach the threshold depolarization that induces an action potential. Clearly, stimulation of muscle contraction by a motor neuron requires the nearly simultaneous release of acetylcholine from numerous synaptic vesicles.

All Five Subunit in the Nicotinic Acetylcholine Receptor Contribute to the Ion Channel
The non-selective nAChR channel gives an equilibrium (or reversal) potential at 0 mV and its current-voltage relationship is ohmic.

The acetylcholine receptor from skeletal muscle is a pentameric protein with a subunit composition of $\alpha_2\beta\gamma\delta$.

**Figure 1.** I-V relationship of nACR.

The acetylcholine receptor from skeletal muscle is a pentameric protein with a subunit composition of $\alpha_2\beta\gamma\delta$.

**Figure 2.** Subunit composition and location of pore and gate within the $\alpha$ subunit.
The channel opens when the receptor cooperatively binds two acetylcholine molecules to sites located at the interfaces of the $\alpha\delta$ and $\alpha\gamma$ subunits. Once acetylcholine is bound to a receptor, the channel is opened virtually instantaneously, probably within a few microseconds. Studies measuring the permeability of different small cations suggest that the open ion channels is, at its narrowest, about 0.65-0.80 nm in diameter, in agreement with estimates from electron micrographs. This would be sufficient to allow passage of both Na$^+$ and K$^+$ ions with their bound shell of water molecules.

Although the structure of the central ion channel is not known in molecular detail, much evidence indicates that it is lined by five transmembrane M2 $\alpha$ helices, one from each of the five subunits. The M2 helices are composed largely of hydrophobic or uncharged polar amino acids, but negatively charged aspartate or glutamate residues are located at each end, near the membrane faces and several serine or threonine residues are near the middle. If a single negatively charged glutamate or aspartate in one subunit is mutated to a positively charged lysine, and the mutant mRNA is injected together with mRNAs for the other three wild-type subunits into frog oocytes, a functional channel is expressed, but its ion conductivity – the number of ions that can cross it during the open state – is reduced. The greater the number of glutamate or aspartate residues mutated (in one or multiple subunits), the greater that reduction in conductivity. These findings suggest that aspartate and glutamate residues – one residue from each of the five chains – form a ring of negative charges on the external surface of the pore that help to screen out anions and attract Na$^+$ or K$^+$ ions as they enter the channel (see Figure 11-36). A similar ring of negative charges lining the cytosolic pore surface also helps select cations for passage.

The two acetylcholine-binding sites in the extracellular domain of the receptor lie ~4 to 5 nm from the center of the pore. Binding of acetylcholine thus must trigger conformational changes in the receptor subunits that can cause channel opening at some distance from the binding sites. Receptors in isolated postsynaptic membranes can be trapped in the open or closed state by rapid freezing in liquid nitrogen. Images of such preparations suggests that the five M2 helices rotate relative to the vertical axis of the channel during opening and closing.

**Synaptic transmission**

Excitable cells communicate with each other by a process called synaptic transmission. This occurs at specialized junctions called synapses connecting the communicating cells. There are two types of synapses: electrical and chemical. Electrical synapses are formed when cells are connected by structures called gap junctions. Gap junctions contain ion channels that permit ion currents to flow between cells, so a depolarizing current in one cell is easily transmitted to its neighbors. Such structures are prominent in cardiac muscle and visceral smooth muscle where they coordinate excitation and contraction over an entire organ. However, most communication between neurons, and between neurons and skeletal muscles occurs through chemical synapses. The neuromuscular junction, important in its own right, is one of the more thoroughly studied chemical synapses and is prototypical of many other synapses.
Structure of neuromuscular junction

A. *From Spinal Cord to Muscle Fibers* – Spinal motor neurons have their cell bodies in the ventral horn of the spinal cord. From there they send out long myelinated axon fibers that contact individual muscle fibers (See figure below).

B. *Motor End Plate* – The fibers then lose their continuous myelin sheath and further branch making synaptic contacts with each muscle cell. The synaptic region is called the motor end plate. There is a distinct space between the presynaptic terminal axon and the postsynaptic sarcolemma, this is called the *synaptic cleft* (see figure below). In a region of the presynaptic axon called the *active zone*, the cytoplasm contains clusters of dense synaptic vesicles. Opposite each of the active zones the postsynaptic muscle membrane invaginates forming *junctional folds*. The junctional folds contain a high density of the postsynaptic acetylcholine (ACh) receptors. The synaptic cleft also contains a matrix of fibrous material to which molecules of the enzyme acetylcholinesterase (AChE) are bound (see figure 3).

![Figure 3](image-url)
End plate potential (epp)

A. *Sequence of events leading to ACh release* – ACh is the excitatory neurotransmitter at the neuromuscular junction. The events leading to its release have been well-researched using a preparation consisting of a few skeletal muscle fibers with the terminal axon of the motor neuron still attached to the muscle (i.e. the motor end plate region is left intact). The isolated nerve-muscle preparation (see Fig. 2) is immersed in a bath of oxygenated Ringer’s solution (an isotonic solution containing the major ions found in the extracellular compartment). A stimulator (a current source that can deliver depolarizing current to the nerve at given intensities and frequencies) makes contact with the motor axon, and a microelectrode impales the muscle cell allowing for the measurement of muscle cell intracellular potentials. The following events (see Fig. 5, part A) are recorded when the axon is stimulated:

![Figure 4](image)
1. An action potential travels down the axon to the presynaptic terminus.
2. This causes the release of ACh from the presynaptic neuron.
3. ACh then diffuses across the synaptic cleft and binds to ACh receptors on the postsynaptic muscle cell membrane.
4. This leads to an action potential recorded on the postsynaptic side (i.e. the muscle cell). Note in part A in the Figure there is a time delay (about 1 msec) between the application of the stimulus to the axon and the first recorded change in potential in the muscle. This delay is the time needed to propagate the action potential to the terminal axon plus the time needed for ACh to diffuse across the synaptic cleft (a distance of about 50 nm). Since the action potential propagates along the axon at a rate of about 20 m/sec, virtually all of the synaptic delay is in the time required for exocytosis and diffusion of ACh. The synaptic clefts can’t be much wider than 50 nm. If they are, the synaptic delay becomes intolerably long, and smooth neuromuscular transmission is impaired.

B. Separating the EEP from the Action Potential – Careful examination of the postsynaptic potential changes shows 2 components:

1. A slowly rising depolarizing potential between -90 mV (muscle resting potential) and about -50 mV that begins to plateau at -50 mV. This is essentially the EPP.
2. Above -50 mV the slope of the potential increases and an action potential occurs. So -50 mV is approximately the threshold potential for the muscle action potential.

The question then becomes: How can the EPP be separated from the action potential, so it can be better-studied? A specific blocker of the EPP is needed. A plant alkaloid mixture called curare contains such a blocker. If curare is ingested or gets into a wound, it can cause skeletal muscles to become paralyzed. The active
substance in the mixture is D-tubocurarine. It acts by competing with ACh for ACh receptor binding sites. In the above figure (part B) a low dose of curare was added to the bath before stimulating. We note that the EPP rises more slowly, but still manages to reach threshold, so an action potential follows. However, at a higher dose, the EPP is further reduced, so it fails to reach threshold (part C). Note curare blocks the EPP but not action potentials per se.

C. Propagation of the EPP—The EPP arises as a consequence of ACh binding to its postsynaptic receptor, so it originates only at the neuromuscular junction. Its “job” is to bring the sarcolemma to threshold potential so action potentials can propagate over the muscle, the precursor event in the process of muscle contraction. In curare-treated muscles the subthreshold EPP behaves like any electrotonic potential. It has its maximum amplitude at the postsynaptic end plate and declines exponentially with distance from the synaptic region.

Mechanism of action of ACh

A. Sequence of Events
1. Nerve action potential reaches the motor neuron presynaptic terminus.
2. ACh is released into the synaptic cleft.
3. ACh diffuses to the postsynaptic membrane where it binds to ACh receptors
4. The permeability of the postsynaptic end plate membrane to both Na⁺ and K⁺ increases causing the postsynaptic membrane to depolarize. This is the EPP and has a peak depolarization of 40-50 mV.
5. The threshold for the initiation of an action potential is reached in the sarcolemma.
6. The action potential propagates along and into the muscle, initiating excitation-contraction coupling.
7. The muscle developments tension.

B. Time Course of the EPP – The EPP rises to a peak within 2-3 msec and then spontaneously decays away. The key factor determining the time course of the EPP is the time course of the changes in ACh concentration in the synaptic cleft. The factors controlling the ACh concentration are: 1) the amount released presynaptically and 2) the rate at which ACh is removed from the synaptic cleft. The factors that determine the rate of removal of ACh are: 1) diffusion away from the synaptic cleft and, 2) more importantly chemical breakdown of ACh mediated by AChE.

C. Effect of AChE on EPP – The importance of AChE in removing ACh from the synaptic cleft is best appreciated when its effects are inhibited. Blockers of AChE activity, such as physostigmine or neostigmine, cause an EPP of greater magnitude and duration and thus potentiate the effects of ACh. That is the reason these anticholinesterases are used to potentiate neuromuscular transmission in diseases such as myasthenia gravis.
Ionic Basis of the EPP – The ACh receptors (AChR) are ligand gated ion channels. The ligand is ACh. When ACh binds to ACh receptors the channel permeability increases to both Na\(^{+}\) and K\(^{+}\), i.e. the AChR is clearly not the same as the voltage gated Na\(^{+}\) channel. The permeability of the AChR is, therefore, unaffected by TTX. The key experiments in proving that the AChR functions as a nonselective monovalent cation channel were done by Sir Bernard Katz and his colleagues. They formed the following hypothesis: If the AChR channel is nonselective between Na\(^{+}\) and K\(^{+}\), then in the motor end plate the membrane potential should go toward approximately 0 mV when ACh binds to the AChRs. They used the isolated nerve-muscle preparation (see above), but with an additional current passing electrode impaling the muscle (see Fig. below).

When no current is passed through the current electrode, the membrane potential is the muscle resting potential of -80 mV. When the stimulator excites the motor axon, the EPP is seen to be a depolarizing potential. Curare is added to prevent action potentials. The peak EPP does not actually reach zero mV because other channels (that are ACh insensitive) prevent the EPP from becoming depolarized to that extent. The key
observation is made when a depolarizing current is passed bringing the membrane potential artificially to 0 mV. *At 0 mV when the nerve is stimulated no EPP is recorded.* This must mean that 0 mV is the reversal potential for the EPP. That can only occur if the AChR channel is passing nearly equal amounts of Na\(^+\) and K\(^+\) in opposite directions. This fact confirms the hypothesis that the AChR channel is nonselective between Na\(^+\) and K\(^+\). This is further confirmed by passing enough current to fix the membrane potential at +30 mV. Stimulation of the nerve causes the potential to again move toward zero, i.e. toward the reversal potential, but this time from the opposite direction.

**Mechanism of ACh release from the presynaptic nerve terminal**

A. *Na\(^+\) and K\(^+\) are not directly involved in the presynaptic release of ACh –* The presynaptic nerve terminal must be depolarized in order for ACh to be released from the nerve terminal. This led to the hypothesis that perhaps slight changes in the Na\(^+\) or K\(^+\) concentration in the presynaptic terminal (as occur naturally during an action potential) were the proximal cause of ACh release. However, it is possible to depolarize the presynaptic nerve terminal by passing current into it, and record postsynaptic EEPs even in the presence of TTX and tetraethylammonium (TEA) which respectively block Na\(^+\) and K\(^+\) channels. This rules out direct roles for Na\(^-\) and K\(^+\) in ACh release.
The effect of Ca\(^{2+}\) on the presynaptic nerve terminal is critical – It had been well known that reducing Ca\(^{2+}\) in the extracellular medium interfered with synaptic transmission. However, the role of extracellular Ca\(^{2+}\) was greatly clarified by Katz and Miledi. They used the nerve-muscle prep, but this time the external bathing medium contained no Ca\(^{2+}\). They positioned a micropipette containing CaCl\(_2\) near the presynaptic terminal. The micropipette also contained a Ag/AgCl electrode into which positive current could be injected at the throw of a switch (see Part A of Fig. above). This enabled them to deliver a small quantity of Ca\(^{2+}\) ions into the synaptic region. (When ions are applied this way in a physiological experiment, the method is called iontophoresis). The results are shown in Part B of the figure. In the column labeled “1" the presynaptic nerve was stimulated, but no Ca\(^{2+}\) was allowed to flow into the synapse. The result is no postsynaptic potential (EPP) was recorded. In column 2 a pulse of Ca\(^{2+}\) was allowed to flow onto the presynaptic terminal region just prior to nerve stimulation. The result is that a postsynaptic potential (EPP) was recorded. In column 3 the nerve was first stimulated briefly and then a small quantity of Ca\(^{2+}\) was released into the presynaptic terminal region. The result again was no postsynaptic potential (EPP). These experiments prove that Ca\(^{2+}\) must be
present to permit ACh to be released from the presynaptic nerve terminal. Note that the negative results in column 3 prove that the effect of Ca$^{2+}$ is on the presynaptic side and not the postsynaptic side.

C. *The Ca$^{2+}$ influx hypothesis for transmitter release* – On the basis of the above results Katz and his coworkers proposed that Ca$^{2+}$ must flow into the presynaptic terminal in order for transmitter (ACh in this case) to be released. Normally the extracellular Ca$^{2+}$ concentration greatly exceeds the intracellular concentration, so an electrochemical ion gradient for Ca$^{2+}$ entry exists. However, the Ca$^{2+}$ permeability of the membrane is normally very low. Katz et al. suggested that when an action potential depolarizes the presynaptic terminal region, that voltage gated Ca$^{2+}$ channels become conducting. This increases the Ca$^{2+}$ permeability allowing Ca$^{2+}$ to flow into the terminal. This somehow permits the release of ACh. This hypothesis (which proves to be correct) is summarized in the Fig. below.

![Diagram](image)

**Figure 9.**

D. *Ca$^{2+}$ dependent ACh release occurs in quantal units* – After many years of research we now know that in the presynaptic nerve terminal ACh is “packaged” in vesicles (each containing roughly 10,000 ACh molecules). When the terminal is depolarized Ca$^{2+}$ enters and binds to the outside of the vesicles. This promotes their fusion with the axolemma and the entire contents of the vesicles are emptied into the synaptic cleft. The process is called **exocytosis**. So ACh gets placed into the synaptic cleft in units of 10,000 molecules. These units were originally referred to as “quanta” before the details of exocytosis were fully known. Sir Bernard Katz and his colleagues performed the seminal physiological experiments that led to conclusion that neurotransmitters must be released in quantal amounts. The morphological evidence for exocytosis followed. In effect Katz et al. observed that even when the motor nerve is not being stimulated, low amplitude potentials can be recorded in the postsynaptic region of the muscle. These potentials had a maximum amplitude of about 0.4 mV and occurred roughly every 50 msec. They were unique to the motor end plate region, were diminished in magnitude by curare and enhanced by neostigmine, i.e. they behaved like EPPs except they were much smaller
and they did not require nerve stimulation to be observed. Katz called them *miniature EPPs* or *MEPPs*. The fig below shows traces of MEPPs. In part A. In part B the histogram shows the amplitudes of the MEPPs are normally distributed about a mean value of 0.4 mV. It seems clear that the MEPPs are due to random ACh release. The question now is: how many ACh molecules in the synaptic region are required to produce a MEPP of 0.4 mV? By using iontophoresis Katz et al. were able to add known amounts of ACh to the synaptic cleft. They found that they could get MEPPs much smaller than 0.4 mV. So it was clear that MEPPs of 0.4 mV were not the result of just a few ACh molecules. They were able to deduce that 0.4 mV corresponded to about 10,000 molecules in the volume of the synaptic cleft. So MEPPs were the result of the release of ACh in unit amounts of 10,000, which is about the number of ACh molecules in the average presynaptic vesicle.

**Figure 10.**

E. *The relation between MEPPs and EPPs* – MEPPs occur spontaneously, i.e. with unstimulated nerve. What is their relation to EPPs, which occur following nerve stimulation? Katz’s underlying hypothesis here was that when the motor nerve is stimulated large numbers of ACh quanta were released in a very short time because they were mobilized by Ca$^{2+}$ influx. So in effect the EPP should be the sum of many MEPPs. To try to control
the number of quanta released so they might be counted discretely, Katz et al. used the usual isolated nerve-muscle preparation, but they kept the Ca^{2+} low. The results of the study are shown in the fig below. They stimulated the motor axon and recorded the amplitude of the evoked EPP that followed. They found that on the average the amplitudes of the evoked EPPs were multiples of the 0.4 mV. This meant that indeed the EPP is made up of a sum of 0.4 mV unit-MEPPs. Since under conditions of normal Ca^{2+} concentrations, EPPs are about 50 mV in magnitude, this means that normal depolarization of the nerve causes the rapid release of at least 100 quanta. Since a quantum is now known to be the same as a fused vesicle, we conclude that an EPP is the result of the fusion of 100 or more vesicles with the presynaptic membrane and the release of about 1 million ACh molecules into the synaptic cleft.
Amplitude of the end-plate potential

Figure 11.
Summary Table

<table>
<thead>
<tr>
<th>Properties of Action Potentials and Synaptic Potentials at the Skeletal Neuromuscular Junction</th>
<th>Synaptic Potential (End-Plate Potential)</th>
<th>Action Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changes in membrane conductance</td>
<td>Initiated by ACh</td>
<td>Initiated by depolarization (the end-plate potential)</td>
</tr>
<tr>
<td>During rising phase (initiation)</td>
<td>Simultaneous chemically gated increase in $g_{Na}$ and $g_{K}$</td>
<td>Specific voltage-dependant increase in $g_{Na}$</td>
</tr>
<tr>
<td>During falling phase</td>
<td>Passive decay; diffusion and AChE 10-20 msec</td>
<td>Specific increase in $g_{K}$ and decrease in $g_{Na}$ 1-3 sec</td>
</tr>
<tr>
<td>Duration</td>
<td>10-20 msec</td>
<td>1-3 msec</td>
</tr>
<tr>
<td>Equilibrium potential</td>
<td>Reversal potential close to 0 mV $E_{Na}$ (+55 mV)</td>
<td></td>
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<tr>
<td>Pharmacology</td>
<td>Blocked by curare; enhanced by neostigmine; not blocked by TTX</td>
<td>Blocked by TTX, but not by curare; not affected by neostigmine</td>
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<tr>
<td>Propagation</td>
<td>Propagates with decrement</td>
<td>All-or-nothing</td>
</tr>
<tr>
<td>Other features</td>
<td>No evidence for regenerative action or refractory period</td>
<td>Regenerative rise, followed by absolute and relative refractory periods</td>
</tr>
</tbody>
</table>
Practice questions

1. The end plate potential:
   A. is specifically blocked by tetrodotoxin
   B. is specifically blocked by tetraethylammonium
   C. has a reversal potential close to 0 mV
   D. has a reversal potential of +60 mV
   E. is due to Ca$^{2+}$ influx into the postsynaptic membrane

2. Removing Ca$^{2+}$ from the end plate region:
   A. has no effect on the end plate potential
   B. enhances the acetylcholinesterase activity
   C. inhibits the release of acetylcholine
   D. starts an action potential in the motor end plate
   E. increases the amplitude of miniature end plate potentials.

Answers

1. C (Because the postsynaptic membrane becomes equally permeable to Na$^+$ and K$^+$)
2. C (Exocytosis of ACh vesicles requires a Ca$^{2+}$ influx to cause their fusion with the presynaptic membrane and release of ACh into the synaptic cleft)

APPENDIX

Ca$^{2+}$-permeable channels

There are a number of channels that when gated open by a number of different mechanisms allow Ca$^{2+}$ ions to move down their steep electrochemical gradient into cells. Given the critical importance of intracellular Ca$^{2+}$ as a second messenger, Ca$^{2+}$ entry exerts important effects on cellular function. Three major categories of Ca$^{2+}$ channels are: a) the extracellular ligand-gated Glutamate receptors (in particular the N-methyl D-Aspartate or NMDA type); b) the voltage-gated Ca$^{2+}$ channels and c) Transient Receptor Potential (TRP) channels found in sensory cells. Here we will only briefly mention the NMDA receptor channels.
Two Types of Glutamate-Gated Cation Channels May Function in a Type of “Cellular Memory”

Figure 12. Glutamate receptor activation results in Ca\(^{2+}\) entry into neuronal cells.
The hippocampus is the region of the mammalian brain associated with many types of short-term memory. Certain types of hippocampal neurons, here simply called postsynaptic cells, receive inputs from hundreds of presynaptic cells. In **long-term potentiation** a burst of stimulation of a post-synaptic neuron makes it more responsive to subsequent stimulation by presynaptic neurons. For example, stimulation of a hippocampal presynaptic nerve with 100 depolarizations acting over only 200 milliseconds causes an increased sensitivity of the postsynaptic neuron that lasts hours to days. Changes in the responses of postsynaptic cells may underlie certain types of memory.

Two types of glutamate-gated cation channels in the postsynaptic neuron participate in long-term potentiation. Unlike other neurotransmitter-gated ion channels, both glutamate receptors have four subunits, each containing a pore-lining M2 helix; both are excitatory receptors, causing depolarization of the plasma membrane when activated. Because the two receptors were initially distinguished by their ability to be activated by the non-natural amino acid N-methyl-D-aspartate (NMDA), they are called **NMDA glutamate receptors** and **non-NMDA glutamate receptors**.

As illustrated in Figure 12, non-NMDA receptors are “conventional” in that binding of glutamate, released from the presynaptic cell, triggers their opening. NMDA glutamate receptors are different in two key respects. First, they allow influx of Ca\(^{2+}\) as well as Na\(^+\). Second, and more important, two conditions must be fulfilled for the ion channel to open: glutamate must be bound and the membrane must be partly depolarized. In this way, the NMDA receptor functions as a **coincidence detector**; that is, it integrates activity of the postsynaptic cell – reflected in its depolarized plasma membrane – with release of neurotransmitter from the presynaptic cell, generating a cellular response greater than that caused by glutamate release alone. Once a post-synaptic cell becomes “sensitized”, it takes fewer action potentials in the presynaptic neurons to induce a given depolarization in the postsynaptic neuron; in other words, the synapse “learns” to have an enhanced response to signals from the presynaptic cells.

Opening of NMDA receptors depends on membrane depolarization because of the voltage-sensitive blocking of the ion channel by a Mg\(^{2+}\) ion from the extracellular solution. A small depolarization of the membrane causes the Mg\(^{2+}\) ion to dissociate from the receptor, thereby making it possible for glutamate binding to open the channel. Mutagenesis of a single asparagine residue in the pore-lining M2 helix of the NMDA receptor abolishes the effect of Mg\(^{2+}\), indicating that Mg\(^{2+}\) binds in the channel.

Since activation of a single synapse, even at high frequency, generally causes only a small depolarization of the membrane of the postsynaptic cell, long-term potentiation is induced only when many synapses simultaneously stimulate a single postsynaptic neuron. Thus, the requirements for membrane depolarization explains why long-term potentiation depends on the simultaneous activation of a large number of synapses on the postsynaptic cell.