Question 1: Outline the steps involved in the presynaptic release of neurotransmitter. Why would the closure of a potassium channel in the presynaptic axon terminal change the amount of Ca^{2+} entering and change the amount of neurotransmitter released?

Answer: The membrane voltage changes during an action potential. The opening of voltage-gated sodium channels causes the rising phase, and closing of the sodium channels and the opening of the potassium channels cause the falling phase. In the axon terminal, voltage-gated calcium channels stay open as long as the membrane voltage exceeds a threshold value. The resulting entry of Ca^{2+} stimulates the release of neurotransmitter. The closure of potassium channels in the axon terminal prolongs the presynaptic action potential, widening the falling phase of the action potential. As a result, the voltage gated Ca^{2+} channels stay open longer, admitting more Ca^{2+} into the terminal, resulting in the release of more quanta of neurotransmitter. This is the molecular basis for sensitization, intensifying the response to all stimuli, even ones that previously evoked little or no reaction.

Question 2: Rabbits can be classically conditioned to blink in response to a tone. This is accomplished by repeatedly pairing the tone with an air puff to the eye. Richard Thompson and his colleagues at Stanford University have made the following observations: Learning fails to occur, and the memory is wiped out, if the cerebellum is surgically removed; the air puff activates cells in the inferior olive; the tone activates cerebellar mossy fibers. Using your knowledge of synaptic plasticity in the cerebellum, propose a mechanism for classical conditioning in the rabbit.

Answer: Classical conditioning involves associating a stimulus that evokes a measurable response with a second stimulus that normally does not evoke this response. We know that in

the cerebellum, learning occurs at the Purkinje cell dendrite, where inputs from the parallel fibers and the climbing fibers converge. This is the Marr-Albus theory of motor learning. This circuitry can also mediate classical conditioning of the eye blink response to a tone paired with a puff of air. If we know that the air puff activates cells in the inferior olive, we know that the climbing fiber input to the Purkinje cells will be carrying this information. If we know that tone activates cerebellar mossy fibers, we know that the parallel fibers will be carrying this information, because mossy fibers synapse on granule cells in the cerebellum, which give rise to parallel fibers that synapse on the Purkinje cells. If these two inputs are paired repeatedly, the air puff (stimulating the climbing fibers) and the tone (stimulating the parallel fibers) will cause long-term potentiation at the Purkinje cell dendrite where the inputs converge. LTP results when synaptic stimulation coincides with strong postsynaptic depolarization. The air puff will cause strong postsynaptic depolarization that will potentiate the input from the parallel fibers representing the tone. As a result, input from the parallel fibers alone will be sufficient to cause the response (eye blink), even though the tone previously did not elicit the eye blink.

Question 3: In Figure 25.16, the mechanisms of classical conditioning in *Aplysia* and LTD in the cerebellar cortex are compared. Expand this comparison to include LTP in the hippocampus. What would events 1 and 2 be? How do these signals converge to affect a common intracellular process? How is the synaptic change expressed?

Answer: LTP results when synaptic stimulation coincides with strong postsynaptic depolarization. In the hippocampus, event 1 would have strong stimulation to a bundle of Shaffer collaterals that synapse on a CA1 neuron. Event 2 would be postsynaptic

depolarization of the CA1 neurons, which occurs as a consequence of *many* excitatory synapses active at the same time (unlike the climbing fiber input to the cerebellum). The intracellular process responsible for LTP in the hippocampus is related to the postsynaptic NDMA receptors on the CA1 neurons (which are not present in the cerebellum). NMDA receptors conduct Ca^{2+} when only glutamate binds and the postsynaptic membrane is depolarized enough to displace Mg^{2+} ions that normally clog the channel. Ca^{2+} entry via the NDMA receptor specifically signals when the presynaptic and postsynaptic elements are active at the same time. The synaptic change is usually expressed as a change in the magnitude of the CA1 neuron's EPSP.

Question 4: What property of the NMDA receptor makes it well suited to detect coincident presynaptic and postsynaptic activity? How could Ca^{2+} entering through the NMDA receptor possibly trigger both LTP and LTD in CA1 and neocortex?

Answer: NMDA receptors have a very high affinity for glutamate, so the transmitter remains bound to the receptor for many tens of milliseconds. Both LTD and LTP are triggered by postsynaptic Ca^{2+}entry through the NMDA receptor. The key difference lies in the level of NMDA receptor activation. When the postsynaptic neuron is only weakly depolarized, the partial blocking of the NMDA receptor channels by Mg^{2+} prevents all but a trickle of Ca^{2+} into the postsynaptic neuron. On the other hand, when the postsynaptic neuron is strongly depolarized, the Mg^{2+} block is displaced entirely, and Ca^{2+} floods into the postsynaptic neuron. These different types of Ca^{2+} response selectively activate different types of enzymes. Instead of the kinases that are activated by high $[Ca^{2+}]_i$, modest and prolonged elevations in $[Ca^{2+}]_i$ activate protein phosphatases, enzymes that pluck phosphate groups off

proteins. Therefore, LTP adds phosphate groups, and LTD takes them off. High-frequency stimulation or multiply active excitatory inputs cause LTP by inducing a large elevation of $[Ca^{2+}]$. Low-frequency stimulation and minimal postsynaptic depolarization causes LTD by producing only a small elevation of $[Ca^{2+}]$.

Question 5: In H.M and R.B. (see Chapter 24), destruction of the hippocampus appears to have impaired the mechanism that "fixes" new memories in the neocortex. Propose a mechanism involving CREB explaining why this might be true.

Answer: R.B. had bilateral hippocampal damage as a result of oxygen deprivation during surgery. H.M. had most of his temporal lobes removed in an effort to control his seizures. Both instances resulted in severe anterograde amnesia. One transcription factor that regulates the process of gene expression required for memory consolidation is called the cyclic AMP response element binding protein (CREB). CREB is a protein that binds to specific DNA segments called cyclic AMP response elements (CREs). It regulates the transcription of neighboring genes. CREB-2 represses gene expression when it binds to CRE. CREB-1 activates transcription, but only when it is phosphorylated by protein kinase A. Memory consolidation can be manipulated by manipulating the availability of CREB-1 and -2. A mechanism involving CREB that blocks memory consolidation in patients such as H.M. and R.B. would require a link between the hippocampus and either an increase in CREB-2 or a decrease in CREB-1, or a decrease in CREB-1 phosphorylation. Perhaps CREB-1 phosphorylation depends on activity in the hippocampus. Phosphorylation is an intracellular process that can be initiated by second messengers in response to neurotransmission at G-

protein-coupled receptors. We can hypothesize that hippocampal activity is necessary for CREB-1 phosphorylation and thus, memory consolidation.