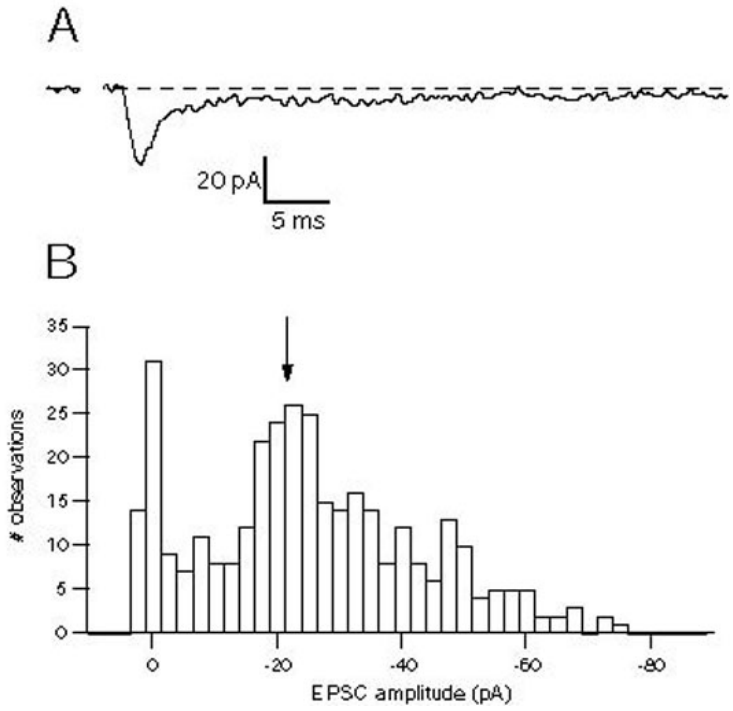
Neuroscience 201A (2016) - Problems in Synaptic Physiology

Question 1:

The record below in **A** shows an EPSC recorded from a cerebellar granule cell following stimulation (at the gap in the record) of a mossy fiber input. These responses are, then, *evoked* by stimulation. The inward current in A is produced by the action of glutamate on AMPA receptors. The histogram in B shows the distribution of many EPSCs, measured at the peak of the average response, to periodic stimulation of the mossy fiber. The arrow in **B** indicates the average peak size of *spontaneously*



occurring synaptic currents recorded from this same granule cell (which have not been illustrated). Assume the following:

a) This granule cell receives only one synaptic input, meaning that only one axon forms synaptic contacts with this cell.
b) The strength of the electrical stimulus delivered to the mossy fiber to produce the responses indicated in A and B was constant for the duration of the experiment and exceeded threshold for eliciting an action potential in the fiber,
c) Release is univesicular, and
d) AMPA receptors are not saturated by a quantum of transmitter.

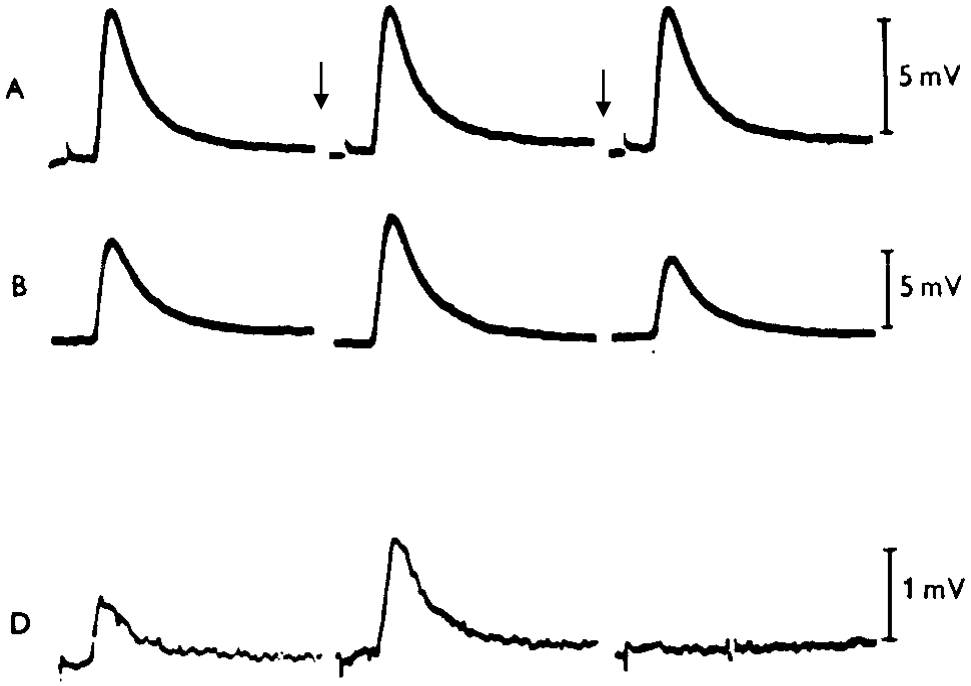
Please answer the following questions:

1. There is a considerable degree of variability from trial-to-trial in the response of the granule cell. The second most important source of fluctuation is in the response of the granule cell to a quantum of transmitter – "quantal variance." What is THE most important source of fluctuation?
2. Provide one possible explanation for quantal variance (which is defined immediately above).
3. Note that some of the responses have values close to 0 pA. What is the quantal content for these responses? Why are these responses not all exactly 0 pA in size?
4. Calculate, ***very*** approximately, the average quantal content for this synapse. Show your reasoning.
5. If the failure rate for this synapse is 0.2 (the fraction of all stimuli that produce no release), if N=4 at this synapse, and if the probability of release, P, is constant across the four releasable quanta, what is P?
6. How would you determine whether release at this synapse were described by the poisson distribution? What would it mean if you were able to show this?

Question #2

Below is a figure depicting four sets (A-D) of three EPPs (muscle EPSPs) recorded consecutively at about 1 Hz. Each of these three EPPs is separated from the next one by a brief gap (arrow in **A**), which represents about a second of time.

Note that in the first set of three responses there is very little fluctuation in EPP size from one event to the next, while in the second set of three responses (**B**) the fluctuation is noticeable. As you know, the size of the EPP in skeletal muscle is usually large enough to reach threshold, and it is therefore impossible to record synaptic responses from muscle fibers without reducing the EPP. For the first set of three events (**A**), EPPs were reduced to subthreshold sizes by adding *d*-tubocurarine (curare). The second set of three responses (**B**) was made subthreshold by the reduction of extracellular calcium concentration and the elevation of extracellular magnesium concentration. In row **D** the average size of the response is reduced further by an additional reduction in calcium (note difference in scale).



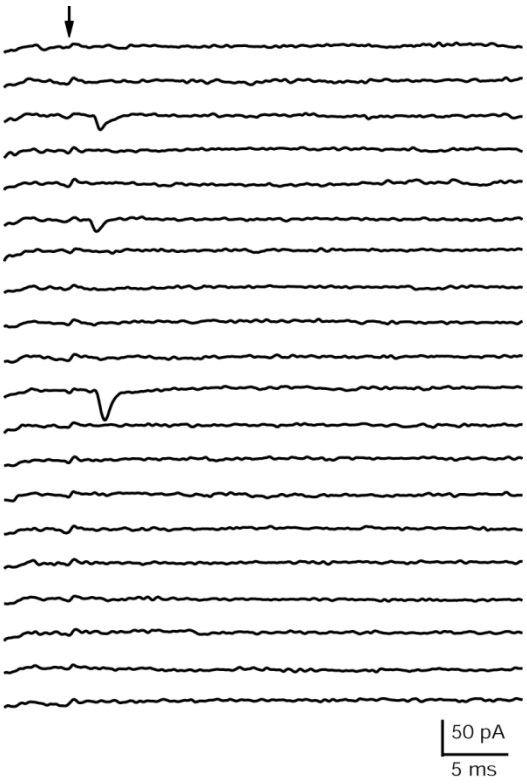
Assume that in normal saline, N=500, P=0.4, and Q=0.5 mV.
Please work the following problems, and show your reasoning.

A. What is m in A?
B. What is m in B?
C. Calculate what fraction of acetylcholine receptors is blocked by the concentration of *d*-tubocurarine used to produce the EPPs in **A**. Assume, for the purposes of your calculation, that the EPP is directly proportional to the number of available acetylcholine receptors.
D. Why do the EPPs fluctuate more in **B** than in **A**? (Ask yourself about the underlying basis of the fluctuation, and then consider how the situation might be different in **A** and **B**, as regards this basis).

Question #3

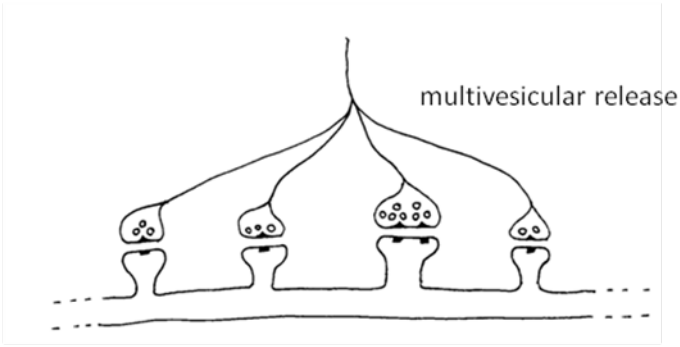
The figure below shows the response of a neuron to 20 successive stimuli, each of which elicits an action potential in a neuron presynaptic to the one from which the recording is made. The traces are displaced each from the next along the vertical axis, for clarity (the displacement is the equivalent of 50 pA, see scale at bottom). The arrow above the top trace indicates the time when the action potential of the presynaptic neuron arrives in the terminal. Following a delay, the neuron sometimes gives a response. The recording is "whole cell," and the cell is voltage clamped at -60 mV.

Calculate as many of the following synaptic parameters as you can from the figure: Q (quantal response), N (number of readily releasable quanta), P (average probability of release), and m (average quantal content). Explain your reasons in calculating these parameters, and if you cannot calculate any of the parameters, list the information that you require to make the calculation.



Question #4:

In the diagram below, two possible explanations for LTP are posed: one involving insertion of AMPA receptors (and the "unsilencing" of synaptic contacts) and the other an increase in P: the probability of release.



before LTP	N	2	2	4	2
	P	0.5	0.5	0.5	0.5
	AMPARs?	no	no	yes	yes
after LTP by AMPAR insertion	N	2	2	4	2
	P	0.5	0.5	0.5	0.5
	AMPARs?	yes	yes	yes	yes
after LTP by increase in P	N	2	2	4	2
	P	0.5	0.5	0.83	0.83
	AMPARs?	no	no	yes	yes

a. What will you measure as quantal content, m, before and after inducing LTP if LTP were produced by AMPA receptor insertion and if LTP were produced by an increase in P? Assume that you are measure m by recording responses (EPSCs) from the postsynaptic cell.
b. Will the results of your measurements (in a, above) allow you to distinguish between the two different mechanisms of LTP? If not, propose an experimental approach that would. In formulating a response, assume that you have no information about the structure of the synapse (what is shown in the cartoon, above) – all that you can do is record "whole cell" from the target neuron and stimulate the presynaptic axon with an extracellular stimulating electrode. You can alter the composition of the solution in which the preparation is bathed and the internal solution in the pipette used for recording from the postsynaptic cell.

Question #5:

A multi-part question about release mechanisms:

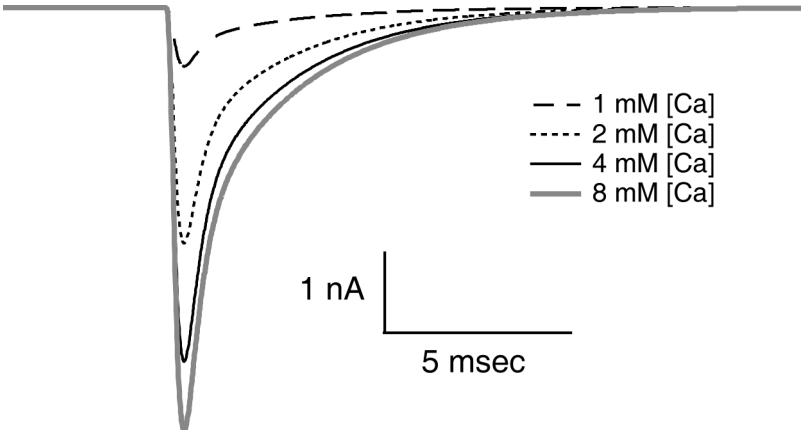
a. What is "univesicular release?"
b. What is the evidence that the principle of univesicular release is applicable at some synapses?
c. Is the evidence for univesicular release influenced by whether or not the postsynaptic receptors are saturated by a quantum of transmitter? Explain why, or why not.
d. Propose two experimental approaches for determining whether postsynaptic receptors are saturated (partially or fully) by a quantum of transmitter.
e. Can you determine whether postsynaptic receptors should be saturated by transmitter from information about the profile of concentration vs. time in the synaptic cleft and by information about the affinity of the receptor for its transmitter? Why or why not?

Question #6

Below are shown four superimposed records of EPSCs elicited from a postsynaptic cell; these were generated by stimulation of an innervating axon at four different concentrations of extracellular calcium. These records were made by recording using a patch pipette in voltage clamp mode from a neuron held at -60 mV.

Assume that the transmitter at this synapse is glutamate.

Assume that release is multivesicular.



What is the principal reason why the response is **steeply** dependent on $[Ca^{2+}]_o$ at lower values of $[Ca^{2+}]_o$?

At high values of $[Ca^{2+}]_o$, the response does not increase in proportion to the increase in $[Ca^{2+}]_o$. The system shows saturation. List two plausible mechanisms for this saturation, one presynaptic (occurring within the terminal) and one postsynaptic (occurring at the surface or within the postsynaptic cell). Propose a set of experiments that would allow you to distinguish between your two plausible mechanisms.

You may use only physiological/biophysical approaches – no molecular biology, biochemistry, or anatomy. Assume that you can record simultaneously from both the postsynaptic cell and the axon terminal that innervates it. Your physiological "rig" has the capacity, as well, to conduct glutamate uncaging experiments.