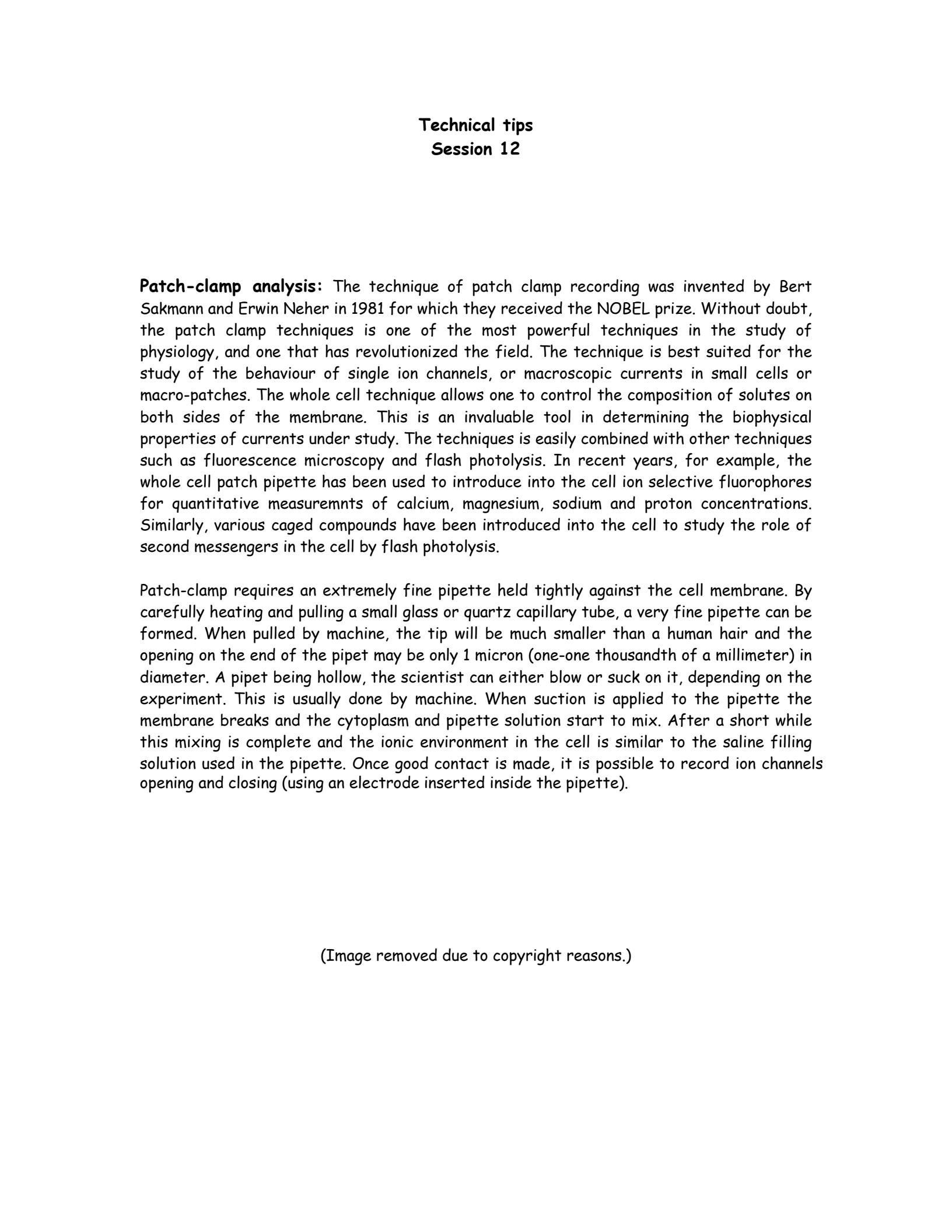
Technical tips
Session 12

Patch-clamp analysis: The technique of patch clamp recording was invented by Bert Sakmann and Erwin Neher in 1981 for which they received the NOBEL prize. Without doubt, the patch clamp techniques is one of the most powerful techniques in the study of physiology, and one that has revolutionized the field. The technique is best suited for the study of the behaviour of single ion channels, or macroscopic currents in small cells or macro-patches. The whole cell technique allows one to control the composition of solutes on both sides of the membrane. This is an invaluable tool in determining the biophysical properties of currents under study. The techniques is easily combined with other techniques such as fluorescence microscopy and flash photolysis. In recent years, for example, the whole cell patch pipette has been used to introduce into the cell ion selective fluorophores for quantitative measuremnts of calcium, magnesium, sodium and proton concentrations. Similarly, various caged compounds have been introduced into the cell to study the role of second messengers in the cell by flash photolysis.

Patch-clamp requires an extremely fine pipette held tightly against the cell membrane. By carefully heating and pulling a small glass or quartz capillary tube, a very fine pipette can be formed. When pulled by machine, the tip will be much smaller than a human hair and the opening on the end of the pipet may be only 1 micron (one-one thousandth of a millimeter) in diameter. A pipet being hollow, the scientist can either blow or suck on it, depending on the experiment. This is usually done by machine. When suction is applied to the pipette the membrane breaks and the cytoplasm and pipette solution start to mix. After a short while this mixing is complete and the ionic environment in the cell is similar to the saline filling solution used in the pipette. Once good contact is made, it is possible to record ion channels opening and closing (using an electrode inserted inside the pipette).



CELL ATTACHED PATCH: When the pipette touches the cell membrane and forms a high resistance seal (~1GOhm), you are in the "cell attached" recording configuration. You do this before making the "whole cell" recording.

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WHOLE CELL RECORDING: When you apply suction to the back of the pipette to break the cell membrane, you enter the "whole cell" recording mode. In this configuration the pipette solution and the cell interior become contiguous.

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The microelectrode is therefore inserted inside the cell with the pipette, which also contains a conducting fluid such as a saline solution. When measuring potential across the plasma membrane this microelectrode is connected to a reference electrode placed in the extracellular fluid and the two are connected to a potenciometer capable of measuring small potential differences (see next two pages to see how ion channel activity is measured).

Overview of the recording set-up: microscope, patch clamp amplifier, computer.

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