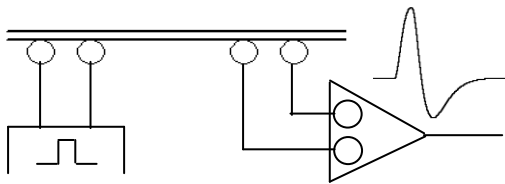


# “SimNerv“: Protocol Form

## Compound Action Potentials (CAPs)

\_\_\_\_\_  
(Name)

Group: ..... Date: .....



\_\_\_\_\_  
(Name of supervisor)

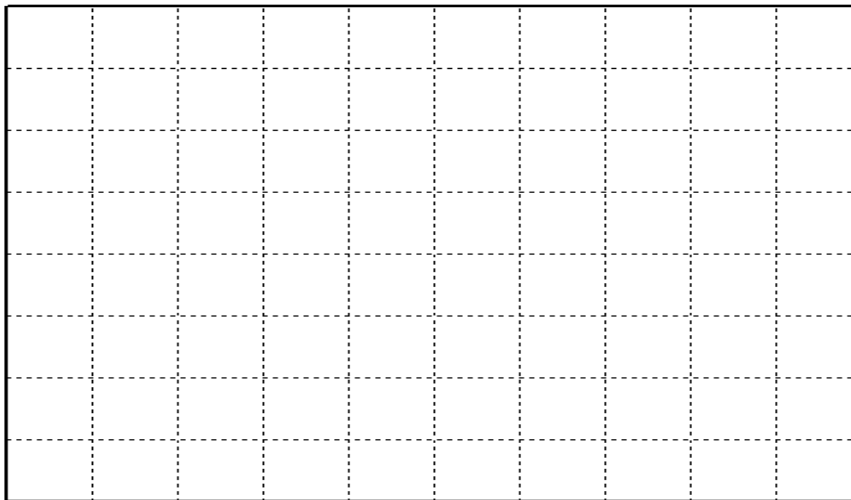
### 1. Specific Features of Extracellular Stimulation and CAP Recordings

#### 1.1 Biphasic and Monophasic Potentials, Differential Amplifier

a) Record a compound action potential of maximum amplitude using a stimulus duration of 1 ms and a sufficiently high stimulus strength. You should see a biphasic potential wave. Set the oscilloscope to the “STORE” mode and try to record a monophasic potential under exactly the same stimulator and oscilloscope settings.

Select a time-base sweep of 0.5 or 1 ms/DIV at the oscilloscope, an appropriate baseline and amplification of channel 2 to display your recordings in good resolution. Please note that the amplitude of the monophasic potential can exceed that of the biphasic potential depending on the distance of your recording electrodes (see task 1.2). of an expectedly biphasic potential wave.

*For the documentation of your results:*



#### Differential Amplifier:

You should be aware in which way the recording electrodes need to be connected to the inverting (-) and non-inverting(+) input of the differential amplifier in order that the biphasic potential wave starts with an upward deflection

Please indicate the in the schematic diagram above the (+) and (-) electrodes.

What happens when the positions of the recording electrodes are exchanged?

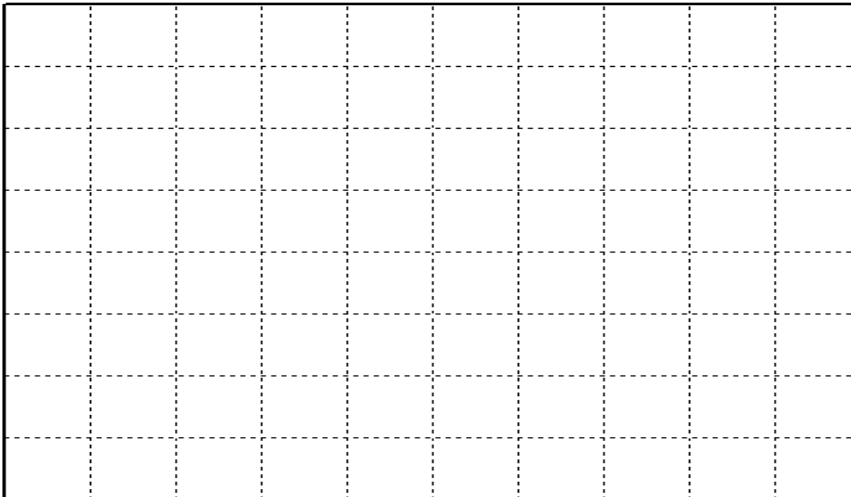
## **1.2 Electrode Positions**

### **1.2.1 Recording Electrodes**

Examine how the compound action potential changes when the distance between the recording electrodes a) is reduced or b) is enhanced

For an easier understanding of these phenomena it is recommended to keep the first recording electrode at its position, only changing the position of the second recording electrode, eventually also to show the monophasic CAP.

*For the documentation of your results:*



### **1.2.2 Stimulation Electrodes:**

Try to find out at which of the stimulation electrodes the action potentials are generated

You can change the electrode position or invert the direction of the current flow ("INVERT" Mode on the stimulator) and also can set a ligature between the two stimulating electrodes. Please note that the direction of the current is given from plus (+) to minus (-), i.e. in the direction of positive charges.

Is the CAP-generating electrode the anode or cathode? \_\_\_\_\_

Is this electrode connected to the plus- or minus-output of the stimulator (positive or negative electrode)? \_\_\_\_\_

Is at this electrode the current flowing from inside to the outside or from the outside to the inside of the membrane? \_\_\_\_\_

Can you imagine how the situation would differ concerning current flow and ionic charges in case of intracellular stimulation (see also SimNeuron)? Should the inserted electrode be the anode or cathode? Can you explain eventual difference on the basis of general principles?

## 2. Stimulus Strength and Duration

### 2.1 CAP-Amplitude as a function of the stimulus strength

Recommended Settings:

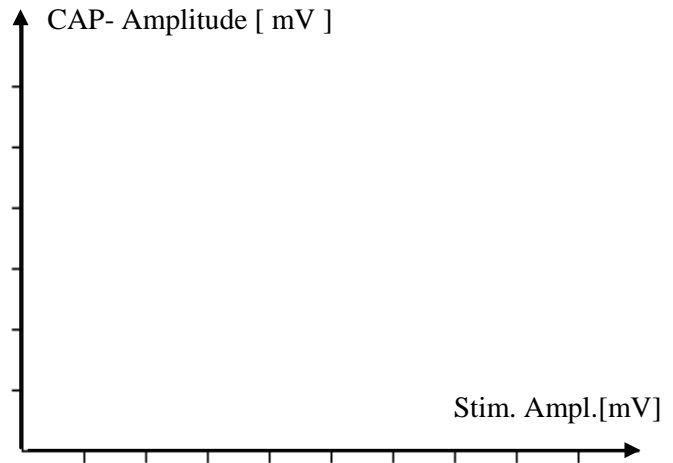
Stimulus Duration: 1 ms. Distance between the recording electrodes: 20 mm

#### 2.1.1 Table of Values

and

#### Diagram:

Stim.-Ampl. [mV]	CAP-Ampl. [mV]



#### 2.1.2 **Minimum (a) and maximum (b) threshold**

a) Stimulus strength that induces a first clearly visible CAP (ca. 0,5 mV): .....mV

b) Stimulus strength at which the maximum CAP Amplitude is reached .....mV

#### 2.1.3 Can this curve agree with the “All-or-None Rule“?

#### 2.1.4 Determine the time-delay from the beginning of the stimulus to the beginning of the CAP

a) at a maximum CAP amplitude (please use clearly supra-maximum stimuli) .....

b) when tiny CAPs are generated (stimulus close to the minimum threshold) .....

*Please apply repeated stimuli at identical settings*

a) Can you explain what processes contribute to the delay and why the delay can change depending on the stimulus strength?

b) You also might have seen that the variability of the responses on identical stimuli is much stronger at lower stimulus amplitudes compared to supra-maximum stimuli (see also 2.2). Can you explain why a much stronger variability, not only concerning the delays but also the CAP amplitudes, can be expected under these stimulus and recording conditions?

## 2.2 The Impact of the Stimulus Duration and the Strength-Duration Curve

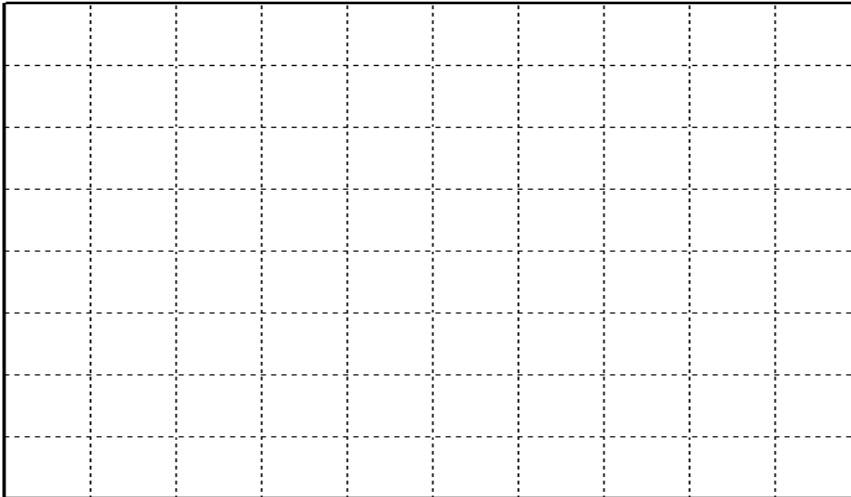
### 2.2.1 Interrelations between Stimulus Duration and Amplitude

Determine the minimum thresholds (CAP of about 0,5 to 1 mV) on a short and longer pulse (e.g. 0.2 und 2 ms).

Stim. Duration[ms]:            0.2        2                    Try to explain the observed differences, at best by means of a thumbnail sketch of the time course of the stimulus induced depolarization.

Stim. Amplitude [mV]        .....        .....

*For the documentation of your results:*



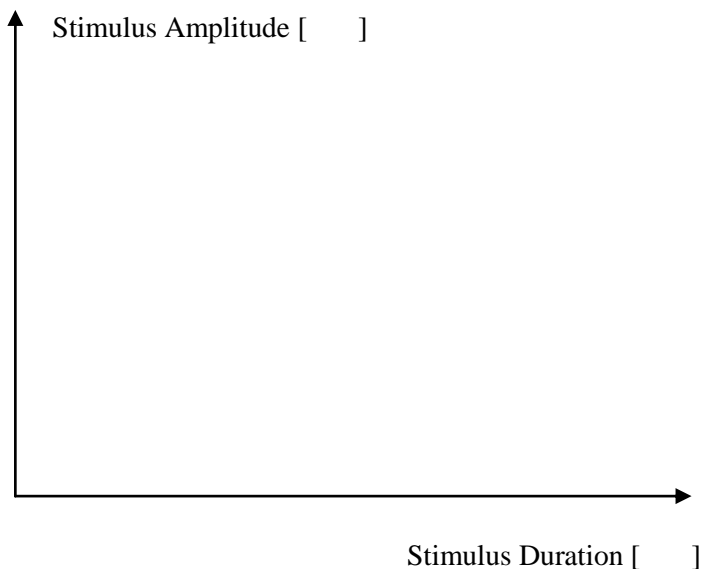
### 2.2.2 Stimulus-Duration Curve

Determine the minimum threshold (CAP of about 0.5 to 1 mV) applying a long stimulus pulse, e.g. of 5 ms. The value of the stimulus amplitude is called "rheobase". Then double the „rheobase" value and change the stimulus duration until you get the same CAP amplitude as before. The value of your stimulus duration is called „chronaxy". With some additional, appropriately selected pairs of values you should be able to reconstruct the complete strength-duration curve. Please indicate „rheobase" and „chronaxy" and try to explain the curve.

#### Table of Values:

Stimulus Duration. [   ]	Stimulus Amplitude [   ]

#### Diagram: Stimulus-Duration Curve



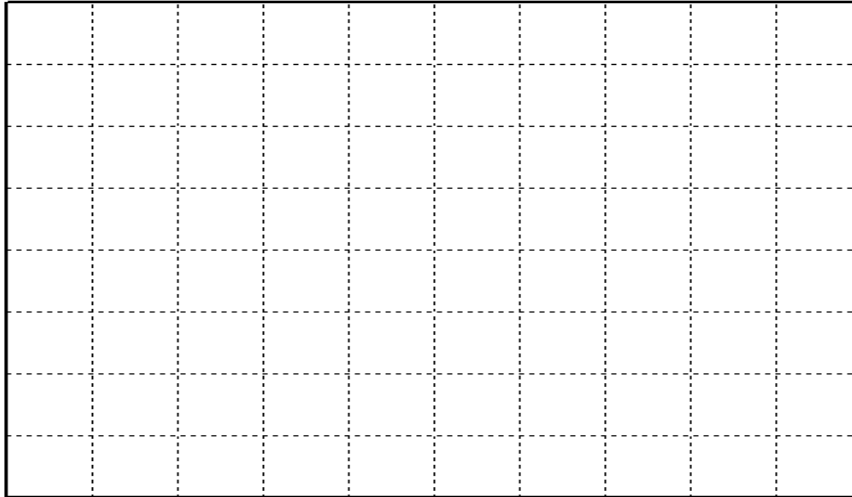
### 3. The Impact of Na<sup>+</sup>-Channel Inactivation

#### 3.1 Refractory Period

##### 3.1.1. Registrations

Apply double pulses (TWIN) of short duration (0.5 ms) and clearly supra-maximum amplitude (.....mV) at varying delays to demonstrate the alterations of the second CAP during the refractory period (STORE mode).

*For the documentation of your results:*



3.1.2 Determine a) the absolute refractory period .....  
b) the relative refractory period .....

3.1.2 Which action potential frequency would correspond to the absolute refractory period? .....

3.1.3 What would be the maximum frequency at which all nerve fibres that contribute to the here recorded CAP still could fire action potentials of maximum amplitude? .....

3.1.4 Please explain which ionic processes lead to the neurons' refractoriness and gradual recovery of their excitability.

3.1.5 Try to draw the time course of an intracellular action potential with the subsequent variation of the trigger threshold.

## **3.2 Anode-Break Potential**

For these recordings it is recommended to adjust the oscilloscope time-base to 1 or 2 ms/DIV which gives you 10 or 20ms over the full display. Set the oscilloscope to the STORE mode.

Use a comparably short stimulus duration of about 1ms and set the stimulus amplitude to a value that a CAP is induced which is slightly smaller than maximum. This is to avoid the occurrence of undesired potentials that may confuse the following recordings.

Take a thread to set a nerve ligature between the stimulating electrodes. The anode should be behind the ligature what you can check pressing the stimulus button again. You should get the same CAP as before.

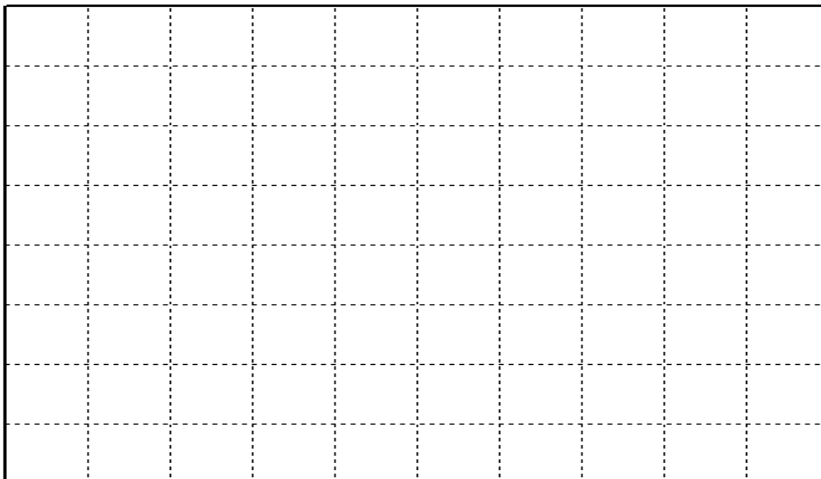
In the next step, exchange the positions of the stimulation electrodes to bring the cathode behind the nerve ligature. This can be done without moving the electrodes by pressing the “invert” button at the stimulator. When the same stimulus is again applied you should no longer see a CAP.

Then increase the stimulus duration, e.g. in steps of 1ms. At certain duration, you should see a small CAP that is increasing with increasing duration although it probably will not reach the amplitude of the potential that is induced by the cathode.

This CAP, for sure, is triggered by the anode and it is apparently generated at the end (break) of the current flow, i.e. when the current circuit is opened. . Therefore it is called “anode-break potential”, or “anode-opening-potential”, in contrast to the “cathode-closing potential” that is generated when the circuit for the current flow is closed.

Please document your recordings and try to explain how action potentials can be generated by the by anode, although current flow at the anode is hyperpolarizing.

*For the documentation of your results:*



**3.2.1** Draw a sketch of the time course of intracellular potential changes and accompanying alterations of the trigger threshold during a long lasting hyperpolarizing current pulse that induces an anode-break potential.

**3.2.2** If you assume intracellular recordings, of what size should the anode-opening potential be in comparison to the cathode-closing potential? Give a short explanation of your opinion.

## **4. Conduction Velocity and Temperature Dependencies**

### **4.1. Nerve Conduction Velocity**

Use a short (1ms) and clearly supra-threshold stimulus to measure the conduction velocity  $v$ , calculating  $v = s/t$  with  $s$  the distance between the cathode and first recording electrode and  $t$  the delay from the beginning of the stimulus to the beginning of the action potential.

Repeat your calculation with application of a smaller stimulus that induces an approximately one third to half maximum CAP.

Document your results and calculate the conduction velocity for both recordings.

*For the documentation of your results:*


#### **4.1.1 Calculation of the conduction velocity**

(Please indicate the time latencies and distances on which your calculations are based)

**4.1.2** What might be the reasons for eventual differences when you stimulate with different stimulus amplitudes?

**4.1.3** Which additional factors would you have to take into account when you stimulate the nerve and record the CAP from the innervated muscle – according to clinical practice?

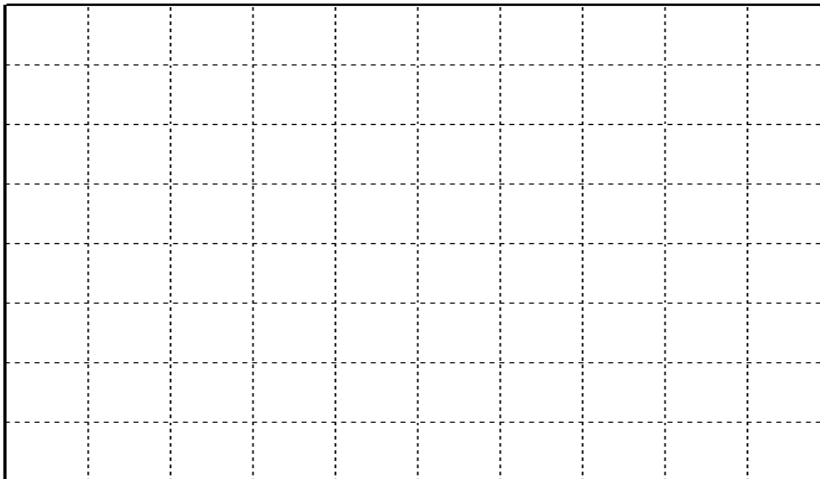
**4.1.4** What stimulation protocol would you use to minimize the error?

**4.1.5** Compare your calculated conduction velocity with those of mammals, including humans.

## 4.1. Temperature Effects

Demonstrate the effects of temperature changes on the nerve conduction velocity setting the recording chamber to different temperatures, e.g. 10, 20, 30 and 40 °C. The electrode positions should thereby keep constant (recommended distance between the recording electrodes: 20 mm).

*For the documentation of your results:*



**4.1.1** Calculate the conduction velocity for two different temperatures and determine how much it changes on average by 1°C temperature change.

**4.1.2** Can you imagine from what membrane mechanisms these temperature dependencies arise?

4.1.3 Can you explain why also the shape of the compound action potential is changed?