5.3. Extracellular electrophysiological recordings

5.3.1. Extracellular potential shifts

Due to the laminar organization of the principal cell populations in the hippocampus, coherent activity of neuronal arrays easily elicits measurable deflections in extracellular potential. These responses offer a useful measure of population activity in hippocampal principal cell population. Field potential electrodes were pulled from borosilicate (GC150F, Clark Electromedical, UK) glass capillaries with the microelectrode puller (Scientific and Research Instruments Ltd, UK). The tip of the electrode was beveled to an approximate diameter of 5 µm. Electrodes were filled with 150 mM NaCl to give them a resistance between 5-20 MΩ. Recording of extracellular potential is also called here “field potential” measurement.

5.3.2. Ion-selective microelectrode recordings

Ion-sensitive electrodes are devices with a Nernst-type voltage sensitivity to changes in activity of a particular ion in a measured solution. The recorded potential shift (E_S) in the ion-selective signal is:

$$E_S = E_C \log \frac{a2}{a1} \qquad (5\text{-}1)$$

, where E_C is the slope (in millivolts) for a 10-fold change in ion concentration, and *a1* and *a2* are the baseline and the shifted activities of the ion, respectively. The general expression for the potential shift as function of the concentration of an ion is:

$$E_S = 2.3 \frac{RT}{Fz} \ln \frac{a2}{a1} \qquad (5\text{-}2)$$

, where *R* is the gas constant, *T* is the absolute temperature (K), *F* is the Faraday constant, and *z* is the valency of the ion (see Table 1). Since E_S reacts to activity shifts in the Nernstian manner, for a 10-fold change in monovalent ion activity, one would expect about a 60 mV shift in the recorded potential (at 32°C). For divalent ions, the ideal potential shift is close to 30 mV. For ion concentrations, activities should be divided by the specific activity coefficient. Free concentration of Ca^{2+} in the normal bicarbonate-buffered physiological solution (stabilized with 5% CO_2) is only about 80% of the total concentration (Heinemann et al., 1977).

Microelectrodes for ion-selective recordings were pulled from double-barreled borosilicate glass (2GC150FS, Clark Electromedical, UK). The non-filamented barrel was silanized by exposure to vapor of TMSDMA (dimethyl-trimethyl-silylamine, Fluka) followed by baking in an oven at 200°C. After silanization, the tip diameter was beveled to 2-10 µm. The filamented barrel was used as a reference electrode, measuring field potential. The silanized barrel was back-filled with a solution which was specific to a measured ion. Then, a short column of the ion-selective sensor was taken into the tip using moderate suction. The same procedure was applied for K^+, H^+, Ca^{2+}, and tetramethylammonium (TMA^+) -selective electrodes. Measurements of the extracellular concentration of the bath-applied impermeable marker TMA^+ were used to study transient activity-induced changes in extracellular space volume. Detailed information about the electrodes is listed in Table 1 below. For the TMA^+ -sensitive sensor, see also Nicholson and Philips (1981).

5.4. Electrophysiological recordings of cellular parameters

5.4.1. Intracellular recordings with sharp electrodes

Microelectrodes with an extremely sharp tip (diameter <0.5 µm) are widely used for recording intracellular potential in mammalian neurons. In this work, the sharp electrode recordings were obtained from pyramidal cells

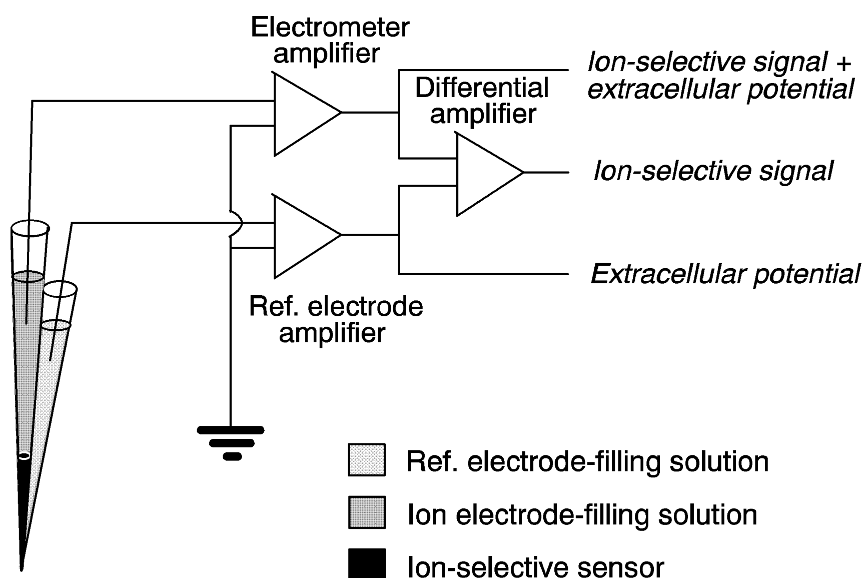

Figure 7. Recording of extracellular ion activity with a double-barreled microelectrode. Ion-selective change is a differential signal. Since the ion-sensitive barrel measures normal extracellular potential in addition to the activity of the specific ion, the reference electrode signal must be subtracted by the differential amplifier.

Recorded ion	Sensor	Filling solution (in mM)	per decade change (mV)	Resistance
K^+	Fluka 60398	150 NaCl, 3 KCl	56-59	5-20 GΩ
H^+	Fluka 95291	100 NaCl, 200 HEPES, 100 NaOH	55-58	10-20 GΩ
Ca^{2+}	Fluka 21048	100 NaCl, 1 $CaCl_2$, 1 HEPES	28-30	15-20 GΩ
TMA^+	Corning 477317	150 NaCl, 3 KCl, 0.5-5 TMACl	55-59	0.5-1 GΩ

Table 1: Data for ion-sensitive electrodes used.

of mature hippocampus (I, IV). The filling solution in the electrodes was *(a)* 0.5 M K-acetate, 5 mM KCl (pH 7.0 with H_2SO_4) for 120-200 MΩ resistance or *(b)* 1 M K-acetate, 1.5 M K-methyl sulphate, 6 mM KCl (pH adjusted to 7.0 with H_2SO_4) for 60-100 MΩ resistance. When passing current to the cell, the potential across the electrode resistance was compensated by a bridge balance (in NPI SECIL amplifier, NPI Electronic Gmbh, Germany). Cell input resistances in resting membrane potential (≤ -60 mV) were 20-100 MΩ.

5.4.2. Whole-cell clamp recordings

In whole-cell patch clamp recording, very low resistance electrodes are used. Intracellular contact is achieved by first sealing the tip of the pipette with the cell membrane and then rupturing the membrane from the patch by applying a slight suction (see Fig. 8). Low resistivity and effective capacitance compensation bring about a fast time constant for the electrode *RC* -circuit. Fast "reactivity" of the electrode makes this technique well-suited for single electrode voltage-clamp recordings. The voltage-clamp enables ion flow across the membrane to be measured as electric current,

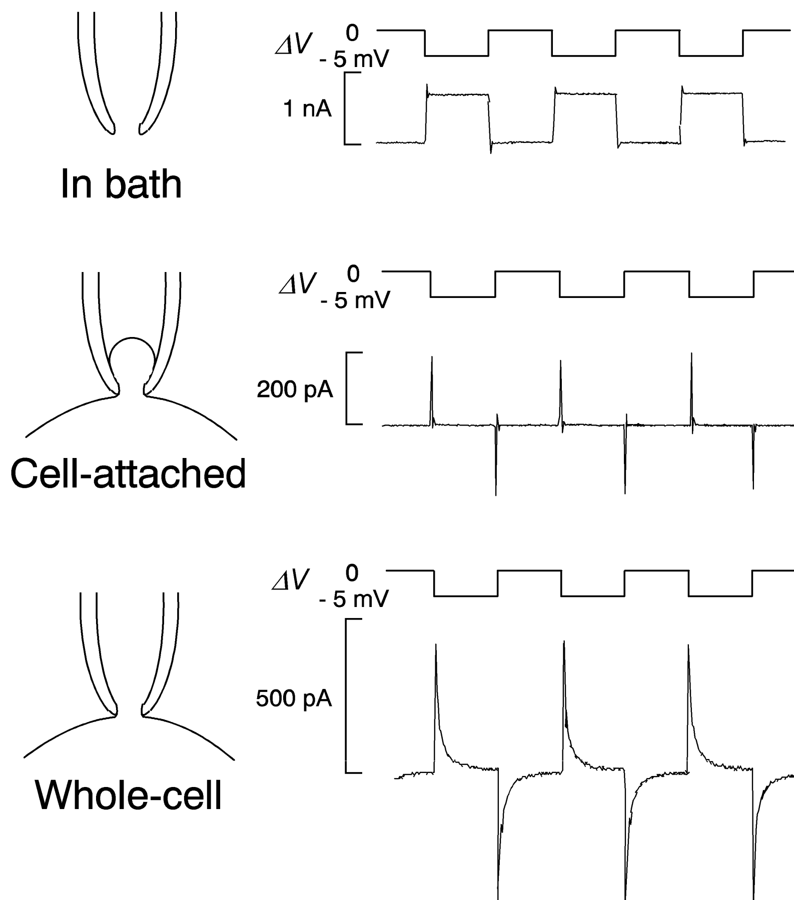

Figure 8. Voltage-clamp of the electrode in bath, in giga seal configuration before rupturing the patched membrane (cell-attached), and voltage-clamp of the pipette and "whole cell". Clamping currents for -5 mV (5 ms) steps illustrated.

whilst membrane voltage is held at a stable value with a feed-back amplifier. Perfusion of the cytoplasm with an electrode-filling solution through the wide tip has a strong influence on intracellular ion concentrations. This was of great value in separation of postsynaptic currents carried by different ions (III, V, VI). We also took advantage of this technique in cell-attached recordings, where action potentials of a single neuron can be measured without altering intracellular ionic content (III, V).

The pipettes were pulled from borosilicate glass capillaries (CG150TF, Clark Electromedical, UK) with a Narishige PP-83 micropipette puller. Electrodes had a resistance of 4-10 MΩ with the filling solutions used listed below (in mM).

1) 140 K-gluconate, 1.5-3 $CaCl_2$, 6 EGTA, 10 HEPES, 2 Mg-ATP (pH 7.0 with NaOH) (V).
2) 125-135 K-gluconate, 1-10 KCl, 2 $Ca(OH)_2$, 5 EGTA, 10 HEPES, 2 Mg-ATP (pH 7.0 with NaOH) (III, VI).

Cells were patched using the "blind method" of Blanton et al. (1989), from the CA3-CA1 area under a conventional binocular light microscope. Input resistance in the gigaseal configuration was 2-10GΩ. The access resistance in the whole-cell configuration was 5-20 MΩ.

Recordings were obtained using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) in continuous voltage-clamp mode.

5.4.3. Gramicidin perforated-patch recordings

In whole-cell patch-clamp recordings, intracellular ionic concentrations are effectively perturbed by dialysis of cytoplasmic contents with the electrode-filling solution. While this offers unique experimental approaches (see *5.4.2.*), the whole-cell clamp technique *per se* cannot be used for measurement of intact cellular potentials. Impalement of sharp microelectrodes into the very small and fragile neonatal cells as well as mature hippocampal interneurons may significantly lower their membrane potential. However, the method of perforated-patch recording can circumvent these problems. Among the commonly used antibiotic ionophores, gramicidin-formed pores are exclusively permeable to monovalent cations (K^+, Na^+ and H^+ in physiological solutions) and small uncharged molecules, but display negligible anion permeability, allowing for patch-clamp recordings, which leave intracellular chloride as well as second messenger systems undisturbed. Gramicidin perforated-patch recordings were used to avoid artifactual changes in membrane potential and E_{GABAA} when effects of $GABA_AR$ activation (which opens chloride and bicarbonate conductance) in neonate hippocampal cells and mature interneurons were studied. Patch pipettes were made from the same glass capillary tubes as in whole-cell patch-clamp recordings. Detailed information about manufacturing gramicidin-perforated electrodes is described in Methods of the original publications (III, VI).

5.5. Pharmacological compounds

Compound	Concentration (µM)	Mechanism of Action	Study
4-aminopyridine (4-AP)	50-100	K^+ channel blocker, increases vesicle secretion	III, IV
Benzolamide (BA)	10	Poorly-permeant inhibitor of carbonic anhydrase	IV
Bicuculline methiodide	10	$GABA_A$ receptor antagonist	II, V, VI
6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX)	20-40	glutamate AMPA/kainate receptor antagonist	III, VI
DL-2-amino-5-phosphono-pentoate (AP5)	40	glutamate NMDA receptor antagonist	I, II, III, IV
Ethoxyzolamide (EZA)	50	membrane permeant inhibitor of carbonic anhydrase	I, II, IV
Ketamine	50	glutamate NMDA receptor antagonist	I, II
Muscimol	0.01-5	$GABA_A$ receptor agonist	VI
6-nitro-7-sulfamoylbenzo[f]quin-oxaline -2,3-dione (NBQX)	10	glutamate AMPA/kainate receptor antagonist	I, II, IV, VI
Pentobarbital (PB)	100	Modulator of the $GABA_A$ receptor channel	II, III, IV
Picrotoxin (PiTX)	100	$GABA_A$ receptor antagonist	I, II, IV

Table 2: Summary of pharmacological substances used in the studies.

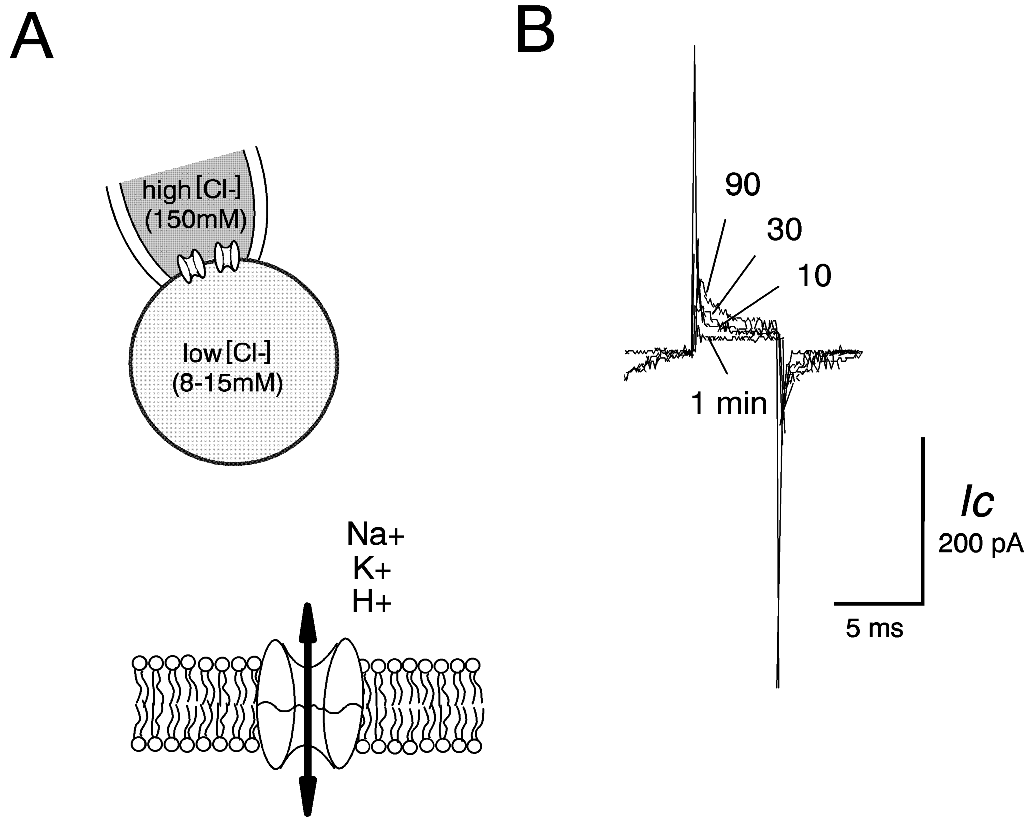

Figure 9. Gramicidin perforated-patch recording. **A)** Since gramicidin pores are impermeable to anions, intracellular recordings do not disturb the transmembrane Cl^- gradient. The current across the perforated patch is carried by small monovalent cations. **B)** A gradual increase in a clamping current (*Ic*) indicates development (min) of gramicidin-perforated conductance through the patched cell membrane.