2 Measurements of electrical signaling by neurons

We will spend today learning about the tools used to measure the activity of neurons. The key idea is the need to make a measurement with high signal-to-noise ratio while minimizing the perturbation to the cell.

2.1 Measurements of intracellular potentials

To probe the spiking characteristics of a cell, we need a means to inject current as well as measure the resultant voltage changes, some of which will lead to spikes. One of the miracles of neuroscience is that one can push a glass micropipet - basically a spear with sharp tip and a hollow core - into a neuron. The core is maybe 300 nm in diameter. In a perfect world one pushes in two micropipets, one to measure the voltage and the other to inject current. The reason for two is that any clogging in the current electrode will not effect the voltage measurement, like "4-wire" measurements in material science.

FIGURE

This is where the fun starts, as the measurement must, on the one hand, not add noise nor drain too much current yet, on the other hand, must not be too resistive. We saw last time that the intrinsic membrane noise is

$$\delta V = \sqrt{\frac{4k_BT\Delta f}{G_{neuron}}}. \tag{2.2}$$

We need to insure that the electrode noise is less than this. The electrode voltage noise is given by the the Johnson noise formula for the series resistance in the electrode, or

$$\delta V = \sqrt{4k_BTR_{electrode}\Delta f}. \tag{2.3}$$

Simply put, we require $R_{electrode} << 1/G_{neuron}$ or that the bulk of the resistance must remain in the cell membrane. It means that the hole in the micropipet cannot be too small. On the other hand, we can only draw a limited amount of current from the cell. This means that the input resistance of the amplifier must be high, , *i.e.*, $R_{amplifier} >> 1/G_{neuron}$ so that, in total

$$R_{electrode} << 1/G_{neuron} << R_{amplifier}. \tag{2.4}$$

In practice, neurons have resistances - at rest - of about 100 MΩ to 10 GΩ so it is simple to satisfy this relation for large cells but not so easy for small cells with a "sharp" micropipet . A modern way to record from a cell that maintains a large pore, and this a small resistance, is with a patch pipet and a technique called "whole cell patch", where you just press the opening against the cell and then suck

to attach the cell then blow to remove the remaining membrane that covers the hole. This provides excellent electrical contact. The price is that proteins, such as those that buffer ions and signaling molecules (which are sometimes the same thing), can diffuse out. A way around this is to have antibiotics (Nystatin) in the pipet so that the drugs punch holes in the membrane that are big enough for ions to go through but too small for proteins. The difficulty here is that the antibiotics mush e diffused in after the pitch is formed. You get the idea - "You can't always get what you want, but if you try real hard then you can get what you need" (M. Jagger). Mick aside, we'll get back to the Nystatin trick at the end when we discuss voltage clamp.

The second part of the story is the input impedance of the amplifier. In practice, even run-of-the mill modern FETs provide resistances of at least $R_{amplifier} \simeq 10^4$ GΩ, so no problem here as a voltage change of even 100 mV would drain 0.01 pA, about 100-times less than the current through a singe channel. For some cases the capacitance of the electrode begins to limit the time response - but we will worry about this at a later date.

FIGURE

A final issue with intracellular recording of voltages is the need to pass current. As noted, the best way is with a separate micro electrode but this is awkward with all but the largest cell and is rarely done with mammalian preparations. One usually passes current through the same electrode, for which the resistance of the electrode causes a voltage drop of $I_{polarization}R_{electrode}$ to appear. To the extent that the electrode remains stable, a serious "if", this offset may be subtracted.

Poking a micropipet into a cell is ideal, not only because you get a true measure of the potential, but because you can fill the cell with a dye after the recording is over and then trace the shape and projections of the neurons. This way you get powerful information that relates, in some sense, structural and response properties. But the method fails, as a practical matter, for recordings that need to last for days and, also as a practical matter, for recordings that need to be stable for hours to days. In this case, we switch to a technique called extracellular recording, is which case record voltage drops that results from currents that flow outside the cell. In order to understand this, we need to develop a few ideas about spatially extended cells (so we can no longer use as model of cells as a ball).

FIGURE

We next estimate the amplitude of the extracellular spike signal. But first, we need to define the electronic length.

2.2 Digression on electrotonic length

The electrotonic distance is the attenuation length of current along the cell. It is a measure of the leakage of the current through the membrane compared to the current that flows along the cytoplasm. For an axon of radius a with membrane thickness L, we can estimate this length by equating the cytoplasmic and membrane resistances, *i.e.*,

$$\rho_{cyt}\frac{\lambda}{\pi a^2} \approx \rho_m \frac{L}{2\pi a \lambda} \tag{2.5}$$

or

$$\lambda = \sqrt{\frac{\rho_m}{\rho_{cyt}}\frac{aL}{2}} = \sqrt{\frac{r_m}{\rho_{cyt}}\frac{a}{2}} \tag{2.6}$$

where the product

$$r_m = \rho_m L \tag{2.7}$$

is denoted as the specific membrane resistance. It has typical values of $r_m = 1$ to 100 kΩcm^2, while the cytoplasm has resistances of order $\rho_{cyt} = 30$ to 300 Ωcm. The spatial attenuation length λ is seen to vary as $\lambda \propto \sqrt{a}$, *i.e.*, a weak change with axonal diameter.

FIGURE

For purposes of dealing with spikes, we need to recall that the specific membrane resistance r_m will decrease during a spike as the membrane becomes leaky, with the greatest leak for sodium ions. This will shorten the electronic length, which is now expressed as

$$\lambda_{AP} = \sqrt{\frac{r_m}{\rho_{cyt}}\frac{1}{r_m G_{Na^+}}\frac{a}{2}} = \frac{\lambda}{\sqrt{r_m G_{Na^+}}} \tag{2.8}$$

where, typically, $G_{Na^+} r_m \simeq 50$ so that $\lambda_{AP} << \lambda$.

2.3 Extracellular measurements of electrical signaling

The first thing we notice is that the extracellular potentials are not the same shape as the intracellular potentials. Can you guess why? As a hint, think of the membrane as a capacitor at high frequencies. What does a capacitor do to a fast signal?

FIGURE

Back to the task of estimating voltages. As a rough estimate - just to get the scales correct - the current flow through the extracellular space is taken to occupy a cylinder of height $Lambda_{AP}$, over which the intracellular current equals the current that leaks to the extracellular space. Approximating the leakage current as uniform along the axon leads to an intracellular current that is given by

$$I_{intracellular} = V_{intracellular} G_{cytoplasm} = \frac{V_{intracellular}}{\rho_{cyt}\frac{\lambda_{AP}}{\pi a^2}}. \tag{2.9}$$

The current that flows outside the axon is taken to do so in a radial manner. We can then calculate the voltage drop between an electrode on a equipotential cylinder (constant radius) just outside the cell, at a radius denoted $X_{measure}$, compared to one far away at a radius further away that we call $X_{reference}$. We recall that the

electric field from a line varies as $E_{extracellular} = K/r$, where K is a constant and r is the radius. Then, with $\vec{E} = -\vec{\nabla}V$, we have

$$V_{extracellular} = -\int_{X_{extracellular}}^{X_{reference}} \frac{K}{r} = K\, ln \frac{X_{reference}}{X_{measure}}. \tag{2.10}$$

We use this to solve for K in the expression for $E_{extracellular}$, *i.e.*,

$$E_{extracellular} = \frac{V_{extracellular}}{r} \frac{1}{ln(X_{reference}/X_{measure})} \tag{2.11}$$

and then write $I_{extracellular}$ in terms of $E_{extracellular}$, recalling that $J_{extracellular} = (1/\rho_{cytoplasm})$ x $E_{extracellular}$ since the conductance outside the cell is about the same as that inside the cell and $I_{extracellular} = J_{extracellular}$ x (Area of cylinder), *i.e.*,

$$I_{extracellular} = \frac{E_{extracellular}}{\rho_{cyt}} 2\pi r \lambda_{AP} = \frac{V_{extracellular}}{\rho_{cyt}} \frac{2\pi\lambda_{AP}}{ln(X_{reference}/X_{measure})}. \tag{2.12}$$

We equate intracellular and extracellular currents to find

$$V_{extracellular} = V_{intracellular} \left(\frac{a}{\lambda_{AP}}\right)^2 \frac{ln(X_{reference}/X_{measure})}{2}. \tag{2.13}$$

We see immediately that $V_{extracellular} << V_{intracellular}$ since $a << \lambda_{AP}$. This can be put in more basic units, which give

$$V_{extracellular} = V_{intracellular} \left(\frac{a\rho_{cyt}}{r_m}\right) (r_m G_{Na^+})\, ln \frac{X_{reference}}{X_{measure}}. \tag{2.14}$$

For the parameters a = 1 μm, $V_{intracellular}$ = 50 mV, $\rho_{cyt} = 2x10^6 \Omega\mu m$, $r_m = 1 \times 10^9 \Omega\mu m$, and $G_{Na^+} r_m = 50$, we have $\lambda_{AP} \simeq 20$ μm. The distances in the logarithmic term may be approximated as $X_{extracellular} \simeq 25$ μm, *i.e.*, the diameter of the electrode, and $X_{reference} \simeq 1$ mm, *i.e.*, the distance to the pia or nearest ventricle, for which $ln(X_{reference}/X_{measure}) \simeq 4$; the logarithmic dependence makes this term relatively insensitive to the exact value of the distances. Thus $V_{intracellular}$ $\simeq 200$ μV. This value is of course approximate. The key issue is that tha ability to measure a signal outside the cell depends on current flow outside the cell, and this is scaled by the electronic length.

The experimental evidence bears out this number. The point of the estimate is to understand the mechanism and thus of course if experiment is close to the theoretically expected value, as a way to improve the measurement. $V_{intracellular} \simeq 200$ μV must be compared against the Johnson noise for a metal electrode, the same formula used above but now with a typical electrode resistance of $1M\Omega$ (or less). The Johnson noise for a 10 kHz bandwidth is $\delta V \simeq 12$ μV. This is the root-mean-square level, so a good rule of thumb is to stay at 5-times this level for an "eye-ball signal-to-noise ratio" of 1 (an RMS signal-to-noise ratio of 5).We are good!

FIGURES

Extracellular signals have additional problems. We often measure contributions from more than one cell and need to "sort" the signals into contributions from individual cells. There is no way to fill the cell of interest with dye. Some tricks - backfiring neurons that project to a given region or using optogenetic activation to fire a specific type of cell - allow aspects of the phenotype of the cell that contributes to the signal to be identified. Of course, the location of the electrode may be estimated with x-rays or more commonly by a lesion made by passing current through the electrode and finding the location with histology.

2.4 Measuring currents

In our understanding of the voltage-dependent currents that form the action potential, and in complete "Hodgkin-Huxley" evaluations, it is imperative to measure what currents flow as a function of potential. This requires a feedback circuit to hold the voltage fixed and to supply whatever current is necessary to accomplish this. These devices are called "Servo's" in Engineering or "Potentiostats" in Chemistry" or a "Voltage Clamp" in Electrophysiology. For this circuit, we can again use an FET op-amp, except that the cell is in the middle of the circuit. The op-amp tries to pass whatever current is necessary from its output in order to hold the level of the "Measuring" voltage as the same value of a reference or "Command" voltage. There are limits to how much current can be passed and to how fast the current can be changed. These effect the measurements. This is also a case where the Nystatin trick is useful, as it allows large currents to be passed without washing out proteins.

FIGURE

2.5 Measuring the current is single channels

The current is a single ion channel - say a sodium channel - is of order 1 pA. How can we measure such a small current? FETs again play a role, as their low input leakage current is small compared to the ion currents. The big issue in making contact with a single channel in a membrane is the seal of the electrode with the membrane. It it is leaky, the leak will cause a Johnson noise. In terms of current, rather than voltage, the Johnson noise is:

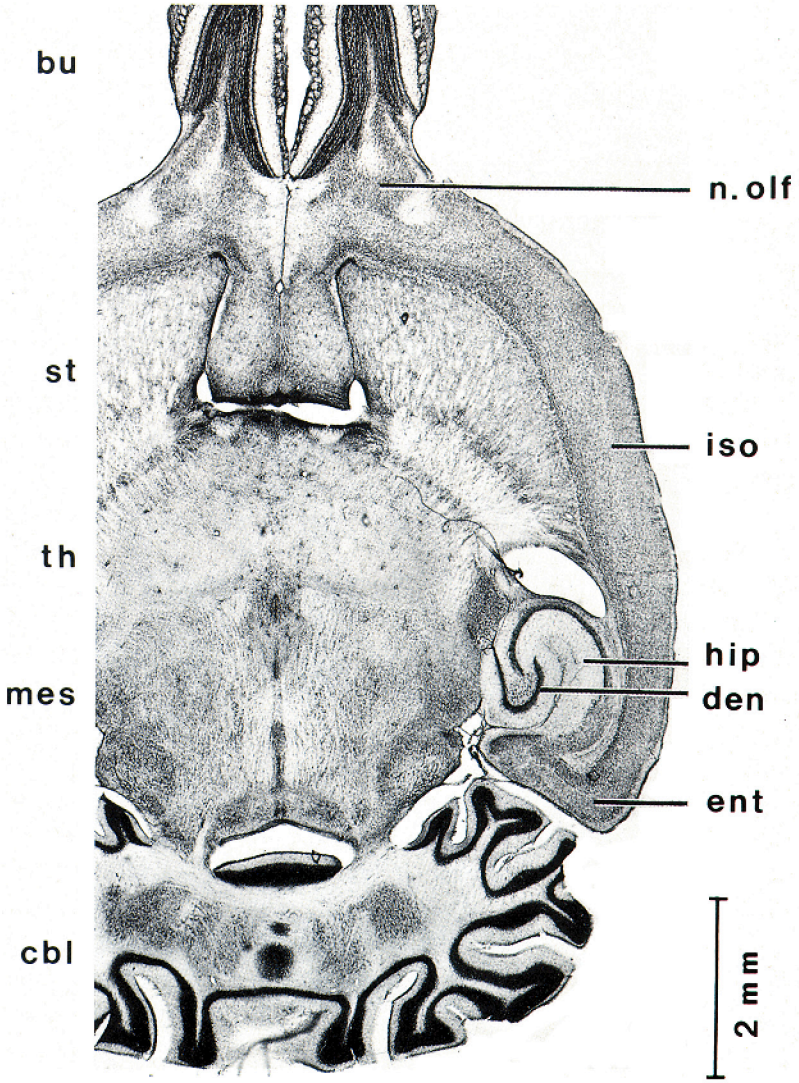
$$\delta I = \sqrt{\frac{4k_B T \Delta f}{R_{patch}}}. \tag{2.15}$$

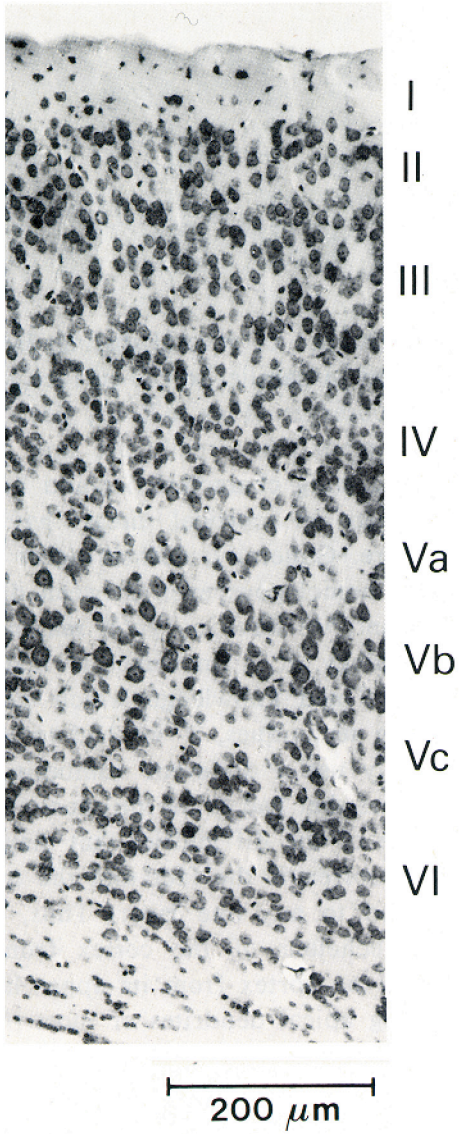
Lets say we want a RMS S/N of 5 with I = 1 pA. Then we find

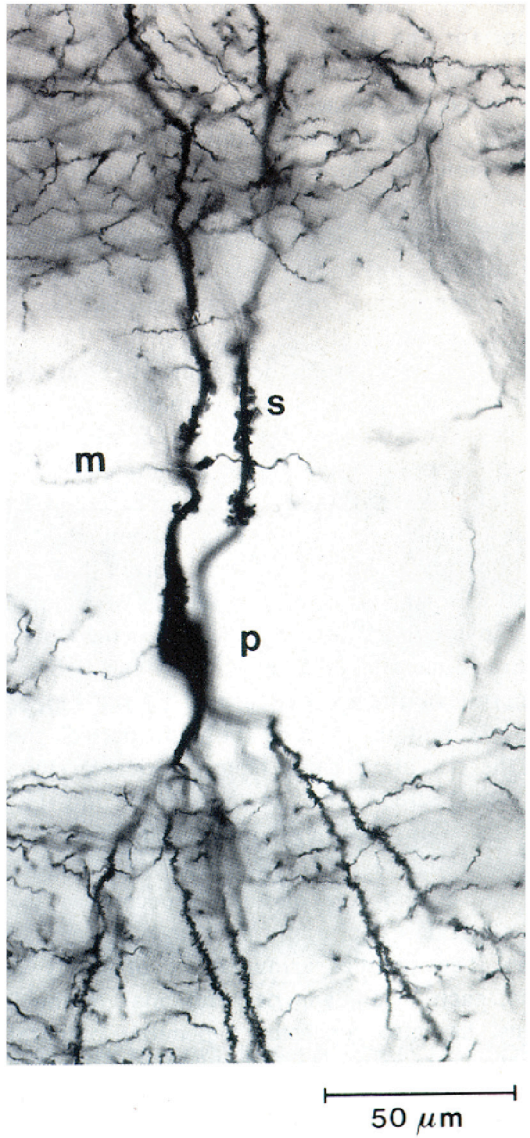
$$R_{patch} > 5 \times \frac{4k_B T \Delta f}{\delta I^2} = 1G\Omega. \tag{2.16}$$

This noise consideration is the origin of the famous "Gigaseal" made famous by Sakmann and Neher. The reasons why phospholipid membranes should stick to glass are not clear, but the phenomenology is a critical tool of neuroscience.

FIGURE







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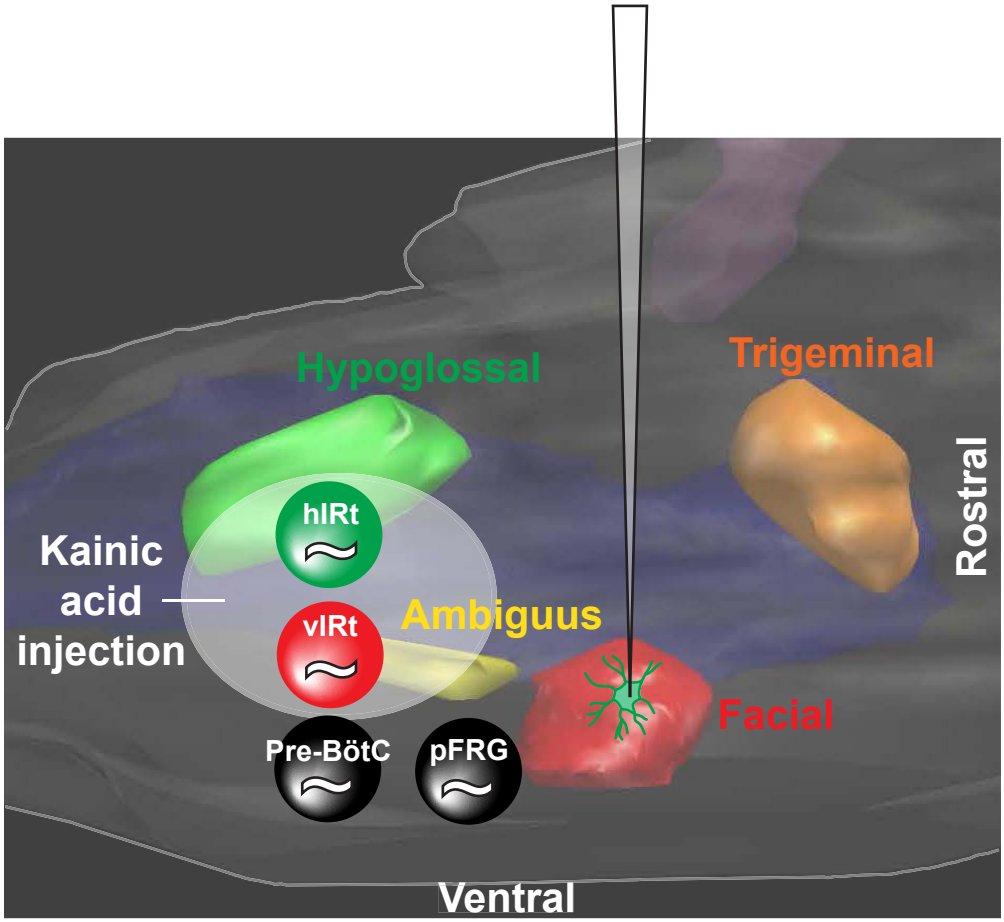
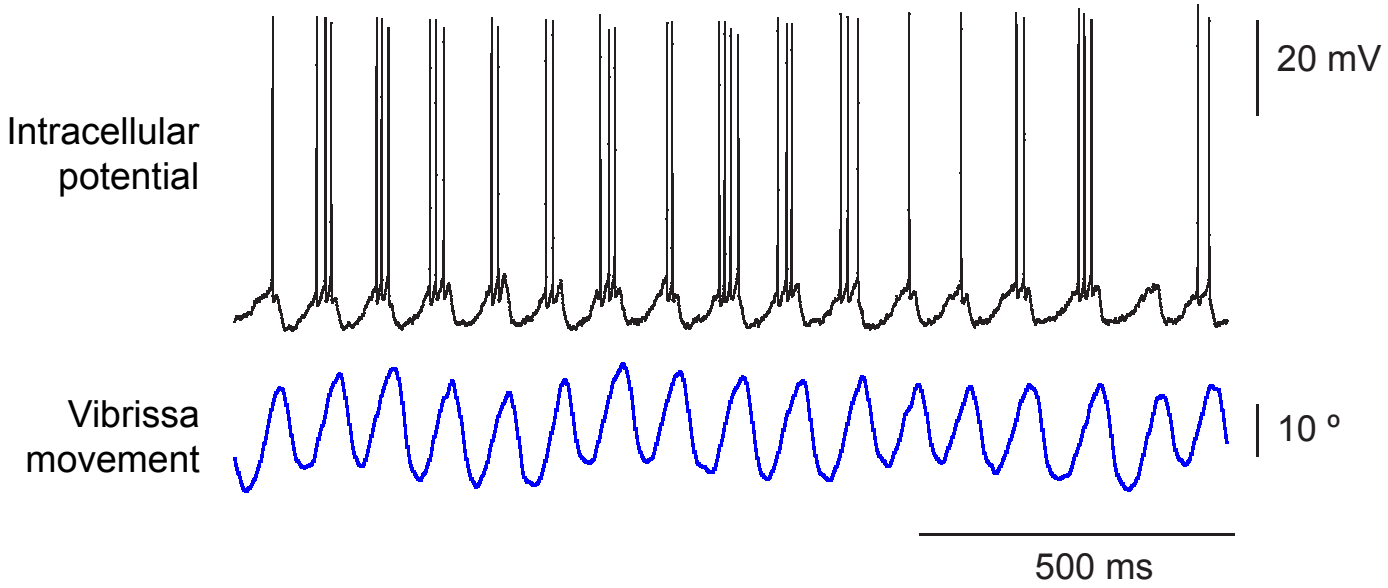
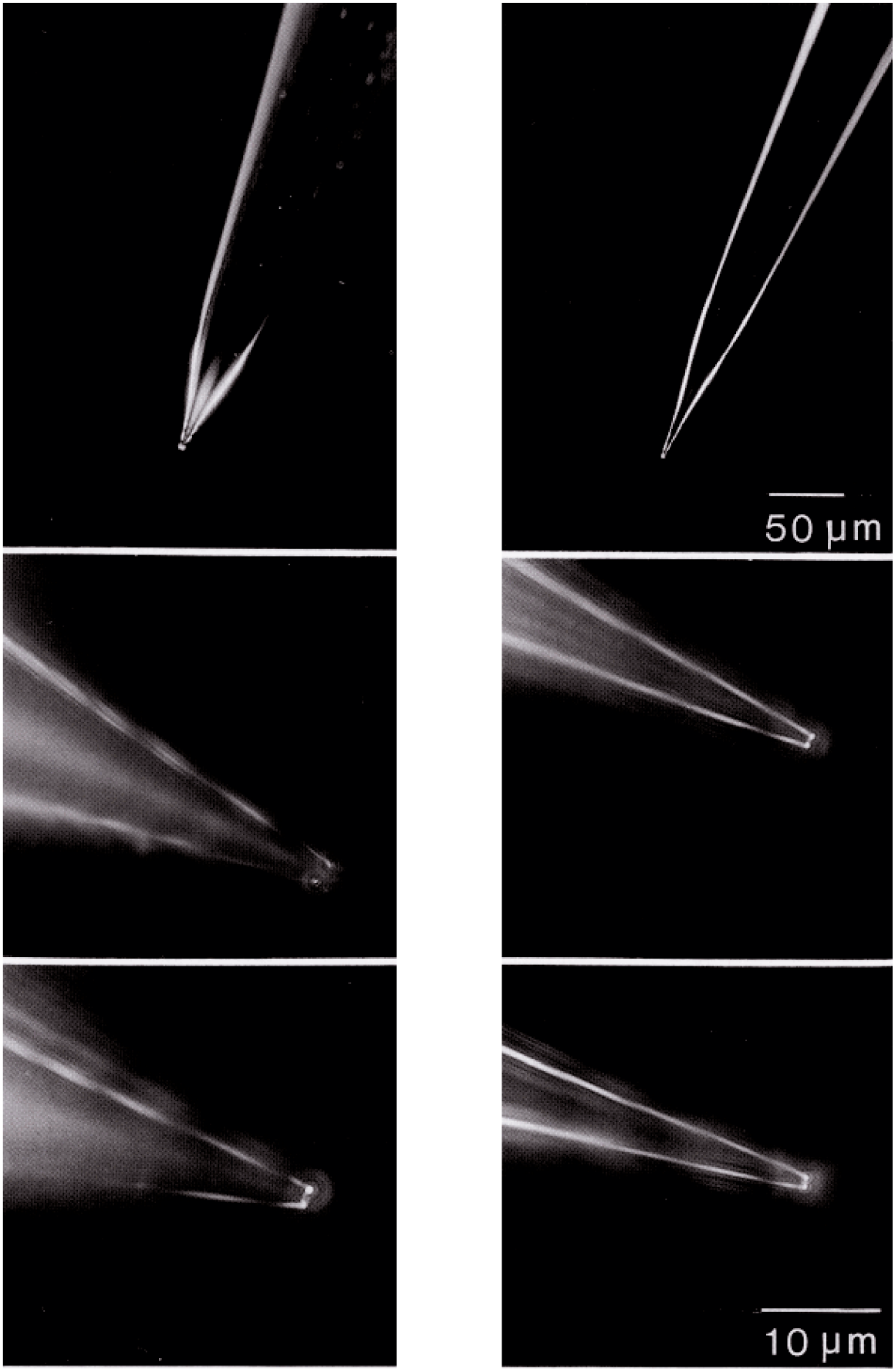

Hypoglossal
Trigeminal
Rostral
hIRt
Kainic
acid
injection
vIRt
Ambiguus
Facial
Pre-BötC
pFRG
Ventral


Intracellular
potential
20 mV
Vibrissa
movement
10 °
500 ms



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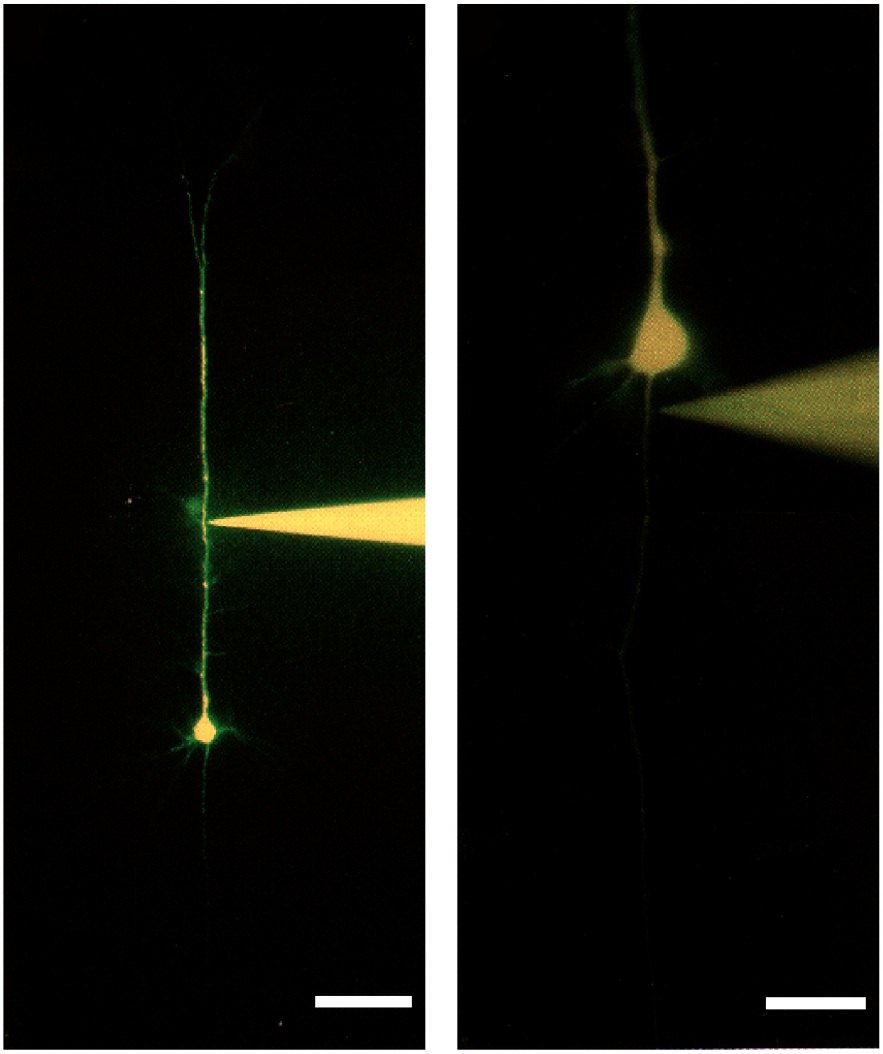

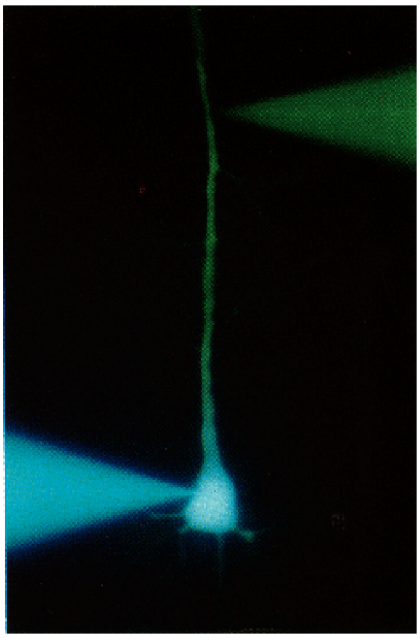

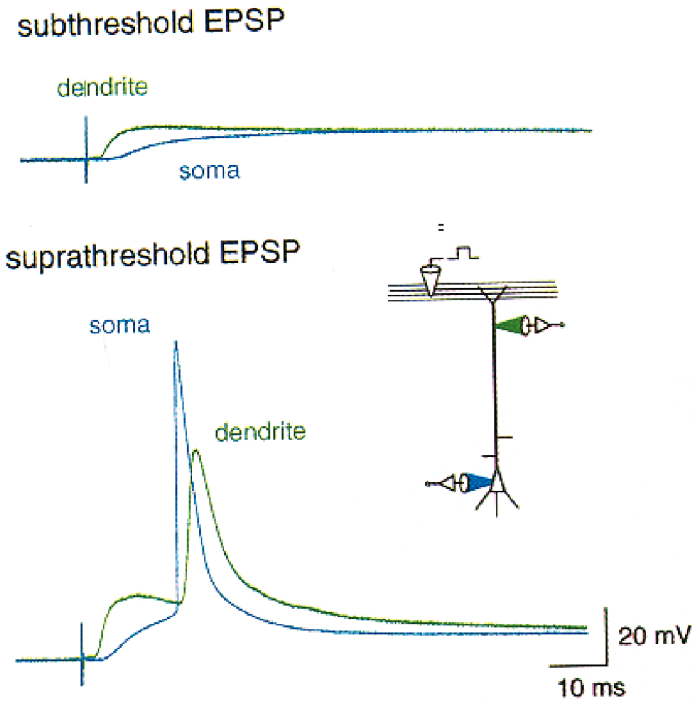

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High-Precision, Low-Noise, Rail-to-Rail Output, 11MHz JFET Op Amp

Check for Samples: OPA140, OPA2140, OPA4140

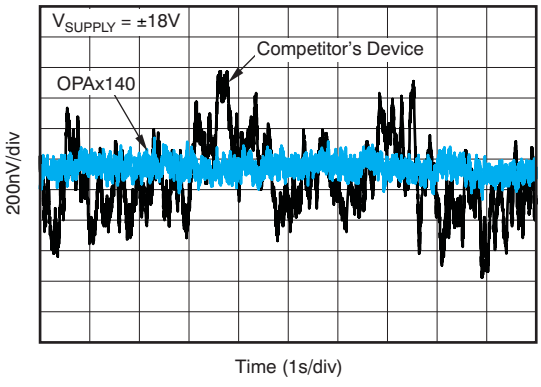
FEATURES

- **Very Low Offset Drift: 1μV/°C max**
- **Very Low Offset: 120μV**
- **Low Input Bias Current: 10pA max**
- **Very Low 1/f Noise: 250nV$_{PP}$, 0.1Hz to 10Hz**
- **Low Noise: 5.1nV/√Hz**
- **Slew Rate: 20V/μs**
- **Low Supply Current: 2.0mA max**
- **Input Voltage Range Includes V– Supply**
- **Single-Supply Operation: 4.5V to 36V**
- **Dual-Supply Operation: ±2.25V to ±18V**
- **No Phase Reversal**
- **Industry-Standard SO Packages**
- **MSOP-8, TSSOP, and SOT23 Packages**

APPLICATIONS

- **Battery-Powered Instruments**
- **Industrial Controls**
- **Medical Instrumentation**
- **Photodiode Amplifiers**
- **Active Filters**
- **Data Acquisition Systems**
- **Automatic Test Systems**

0.1Hz to 10Hz NOISE



DESCRIPTION

The OPA140, OPA2140, and OPA4140 op amp family is a series of low-power JFET input amplifiers that feature good drift and low input bias current. The rail-to-rail output swing and input range that includes V– allow designers to take advantage of the low-noise characteristics of JFET amplifiers while also interfacing to modern, single-supply, precision analog-to-digital converters (ADCs) and digital-to-analog converters (DACs).

The OPA140 achieves 11MHz unity-gain bandwidth and 20V/μs slew rate while consuming only 1.8mA (typ) of quiescent current. It runs on a single 4.5 to 36V supply or dual ±2.25V to ±18V supplies.

All versions are fully specified from –40°C to +125°C for use in the most challenging environments. The OPA140 (single) is available in the SOT23-5, MSOP-8, and SO-8 packages; the OPA2140 (dual) is available in both MSOP-8 and SO-8 packages; and the OPA4140 (quad) is available in the SO-14 and TSSOP-14 packages.

RELATED PRODUCTS

FEATURES	PRODUCT
Low-Power, 10MHz FET Input Industrial Op Amp	OPA141
2.2nV/√Hz, Low-Power, 36V Operational Amplifier in SOT23 Package	OPA209
Low-Noise, High-Precision, 22MHz, 4nV/√Hz JFET-Input Operational Amplifier	OPA827
Low-Noise, Low I_Q Precision CMOS Operational Amplifier	OPA376
High-Speed, FET-Input Operational Amplifier	OPA132

ELECTRICAL CHARACTERISTICS: V_S = +4.5V to +36V; ±2.25V to ±18V

Boldface limits apply over the specified temperature range, **T_A = –40°C to +125°C**.
At T_A = +25°C, R_L = 2kΩ connected to midsupply, V_{CM} = V_{OUT} = midsupply, unless otherwise noted.

PARAMETER		CONDITIONS	OPA140, OPA2140, OPA4140 MIN	TYP	MAX	UNIT
OFFSET VOLTAGE						
Offset Voltage, RTI	V_{OS}	V_S = ±18V		30	120	µV
Over Temperature		**V_S = ±18V**			**220**	**µV**
Drift	**dV_{OS}/dT**	**V_S = ±18V**		**±0.35**	**1.0**	**µV/°C**
vs Power Supply	PSRR	V_S = ±2.25V to ±18V		±0.1	±0.5	µV/V
Over Temperature		**V_S = ±2.25V to ±18V**			**±4**	**µV/V**
INPUT BIAS CURRENT(1)						
Input Bias Current	I_B			±0.5	±10	pA
Over Temperature					**±3**	**nA**
Input Offset Current	I_{OS}			±0.5	±10	pA
Over Temperature					**±1**	**nA**
NOISE						
Input Voltage Noise						
f = 0.1Hz to 10Hz				250		nV_{PP}
f = 0.1Hz to 10Hz				42		nV_{RMS}
Input Voltage Noise Density	e_n					
f = 10Hz				8		nV/√Hz
f = 100Hz				5.8		nV/√Hz
f = 1kHz				5.1		nV/√Hz
Input Current Noise Density	I_n					
f = 1kHz				0.8		fA/√Hz
INPUT VOLTAGE RANGE						
Common-Mode Voltage Range	**V_{CM}**		**(V–) –0.1**		**(V+)–3.5**	**V**
Common-Mode Rejection Ratio	CMRR	V_S = ±18V, V_{CM} = (V–) –0.1V to (V+) – 3.5V	126	140		dB
Over Temperature		**V_S = ±18V, V_{CM} = (V–) –0.1V to (V+) – 3.5V**	**120**			**dB**
INPUT IMPEDANCE						
Differential				10^{13} || 10		Ω || pF
Common-Mode		V_{CM} = (V–) –0.1V to (V+) –3.5V		10^{13} || 7		Ω || pF
OPEN-LOOP GAIN						
Open-Loop Voltage Gain	A_{OL}	V_O = (V–)+0.35V to (V+)–0.35V, R_L = 10kΩ	120	126		dB
		V_O = (V–)+0.35V to (V+)–0.35V, R_L = 2kΩ	114	126		dB
Over Temperature		**V_O = (V–)+0.35V to (V+)–0.35V, R_L = 2kΩ**	**108**			**dB**
FREQUENCY RESPONSE						
Gain Bandwidth Product	BW			11		MHz
Slew Rate				20		V/µs
Settling Time, 12-bit (0.024)				880		ns
Settling Time, 16-bit				1.6		µs
THD+N		1kHz, G = 1, V_O = $3.5V_{RMS}$		0.00005		%
Overload Recovery Time				600		ns

(1) High-speed test, T_A = T_J.

APPLICATION INFORMATION

The OPA140, OPA2140, and OPA4140 are unity-gain stable, operational amplifiers with very low noise, input bias current, and input offset voltage. Applications with noisy or high-impedance power supplies require decoupling capacitors placed close to the device pins. In most cases, 0.1μF capacitors are adequate. Figure 1 shows a simplified schematic of the OPA140.

OPERATING VOLTAGE

The OPA140, OPA2140, and OPA4140 series of op amps can be used with single or dual supplies from an operating range of V_S = +4.5V (±2.25V) and up to V_S = +36V (±18V). These devices do not require symmetrical supplies; they only require a minimum supply voltage of +4.5V (±2.25V). For V_S less than ±3.5V, the common-mode input range does not include midsupply. Supply voltages higher than +40V can permanently damage the device; see the Absolute Maximum Ratings table. Key parameters are specified over the operating temperature range, T_A = –40°C to +125°C. Key parameters that vary over the supply voltage or temperature range are shown in the Typical Characteristics section of this data sheet.

CAPACITIVE LOAD AND STABILITY

The dynamic characteristics of the OPAx140 have been optimized for commonly encountered gains, loads, and operating conditions. The combination of low closed-loop gain and high capacitive loads decreases the phase margin of the amplifier and can lead to gain peaking or oscillations. As a result, heavier capacitive loads must be isolated from the output. The simplest way to achieve this isolation is to add a small resistor (R_{OUT} equal to 50Ω, for example) in series with the output.

Figure 20 and Figure 21 illustrate graphs of *Small-Signal Overshoot vs Capacitive Load* for several values of R_{OUT}. Also, refer to Applications Bulletin AB-028 (literature number SBOA015, available for download from the TI web site) for details of analysis techniques and application circuits.

NOISE PERFORMANCE

Figure 34 shows the total circuit noise for varying source impedances with the operational amplifier in a unity-gain configuration (with no feedback resistor network and therefore no additional noise contributions). The OPA140 and OPA211 are shown with total circuit noise calculated. The op amp itself contributes both a voltage noise component and a current noise component. The voltage noise is commonly modeled as a time-varying component of the offset voltage. The current noise is modeled as the time-varying component of the input bias current and reacts with the source resistance to create a voltage component of noise. Therefore, the lowest noise op amp for a given application depends on the source impedance. For low source impedance, current noise is negligible, and voltage noise generally dominates. The OPA140, OPA2140, and OPA4140 family has both low voltage noise and extremely low current noise because of the FET input of the op amp. As a result, the current noise contribution of the OPAx140 series is negligible for any practical source impedance, which makes it the better choice for applications with high source impedance.

The equation in Figure 34 shows the calculation of the total circuit noise, with these parameters:

- e_n = voltage noise
- I_n = current noise
- R_S = source impedance
- k = Boltzmann's constant = 1.38×10^{-23} J/K
- T = temperature in degrees Kelvin (K)

For more details on calculating noise, see the section on Basic Noise Calculations.

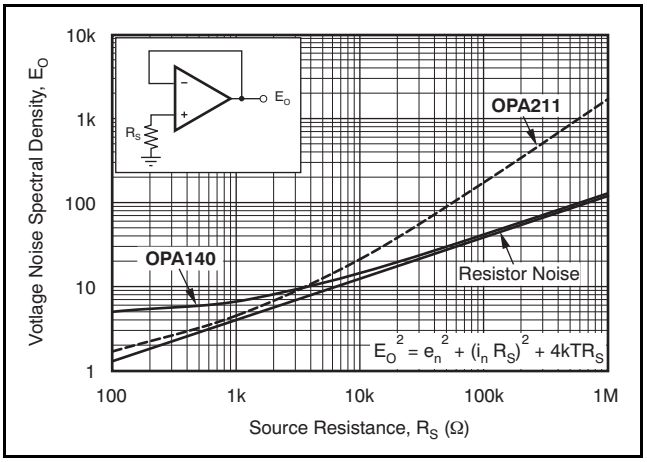
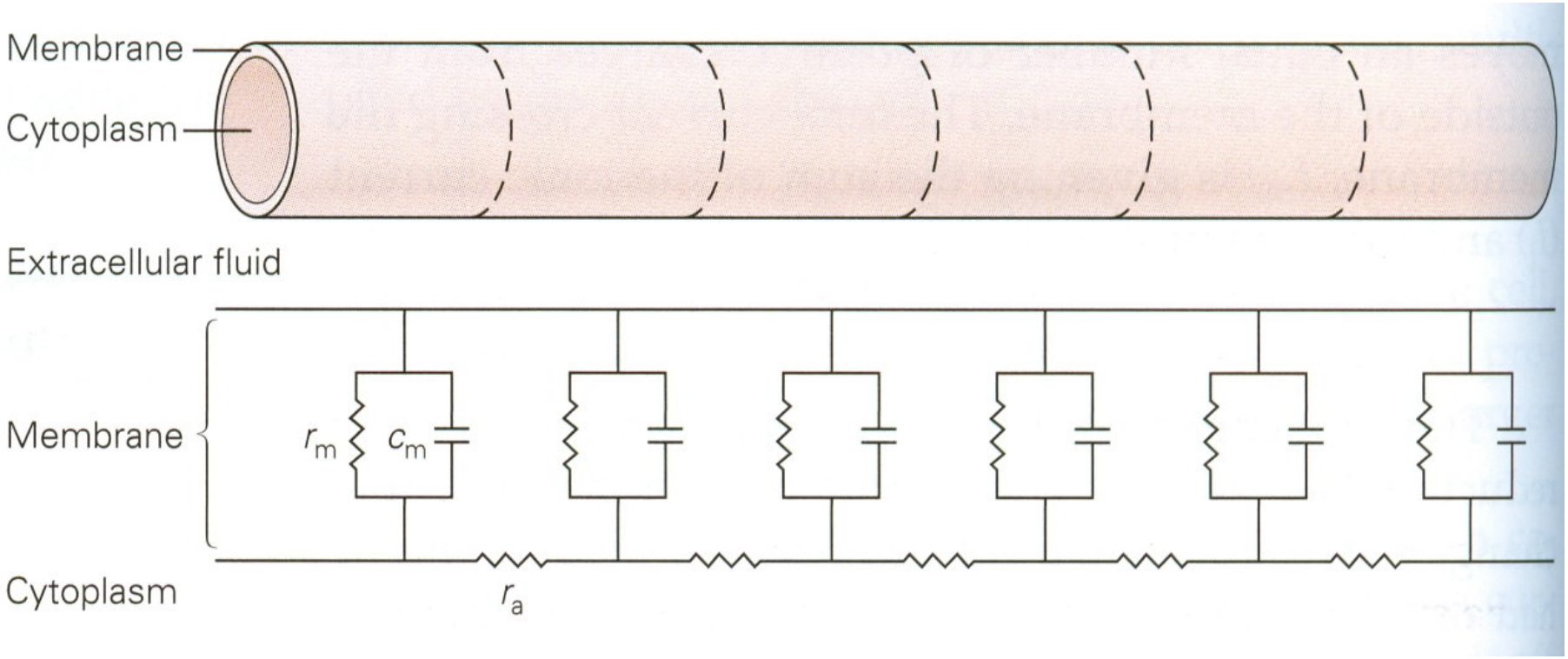
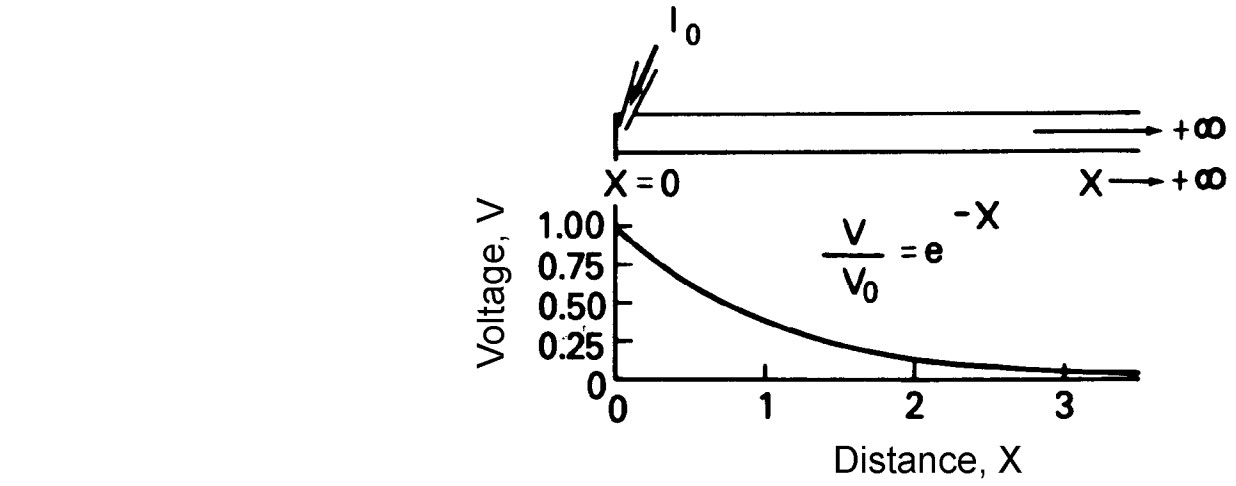


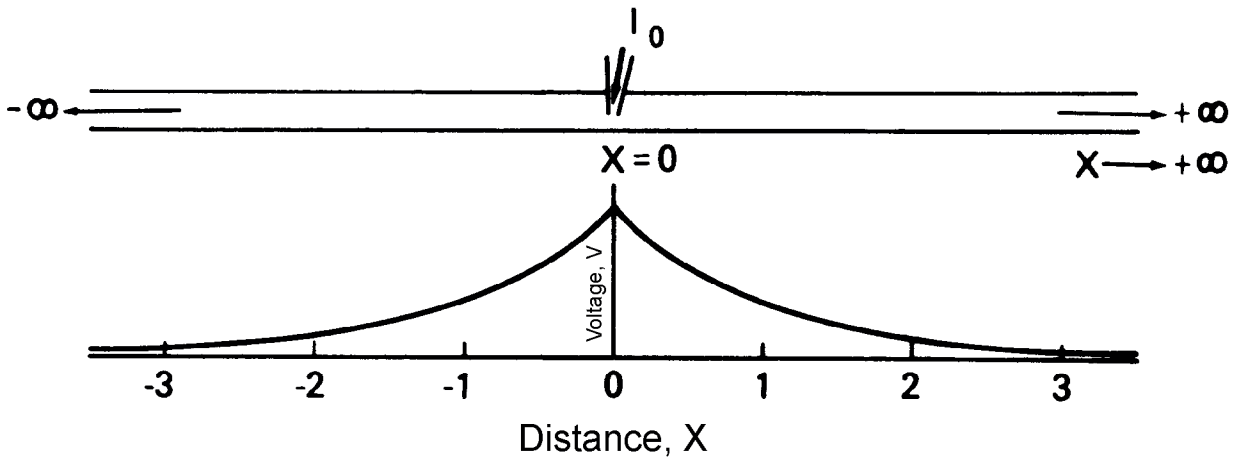
Figure 34. Noise Performance of the OPA140 and OPA211 in Unity-Gain Buffer Configuration

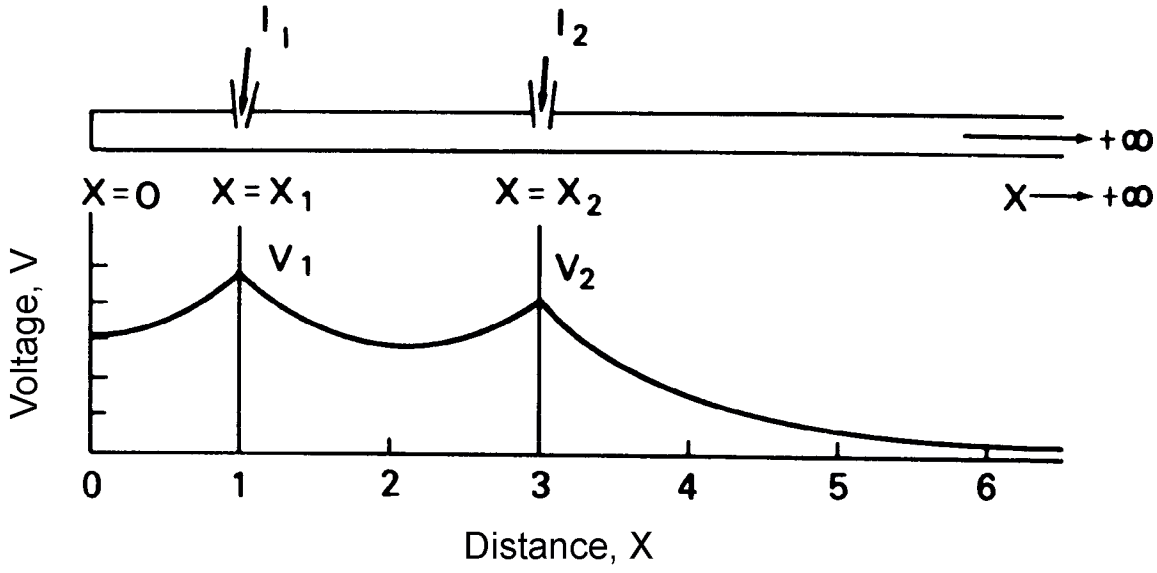
Passive Properties: Long Cable

- Describe cable as a series of RC circuits
- Allow the thickness of each segment $\Delta x \rightarrow$ zero



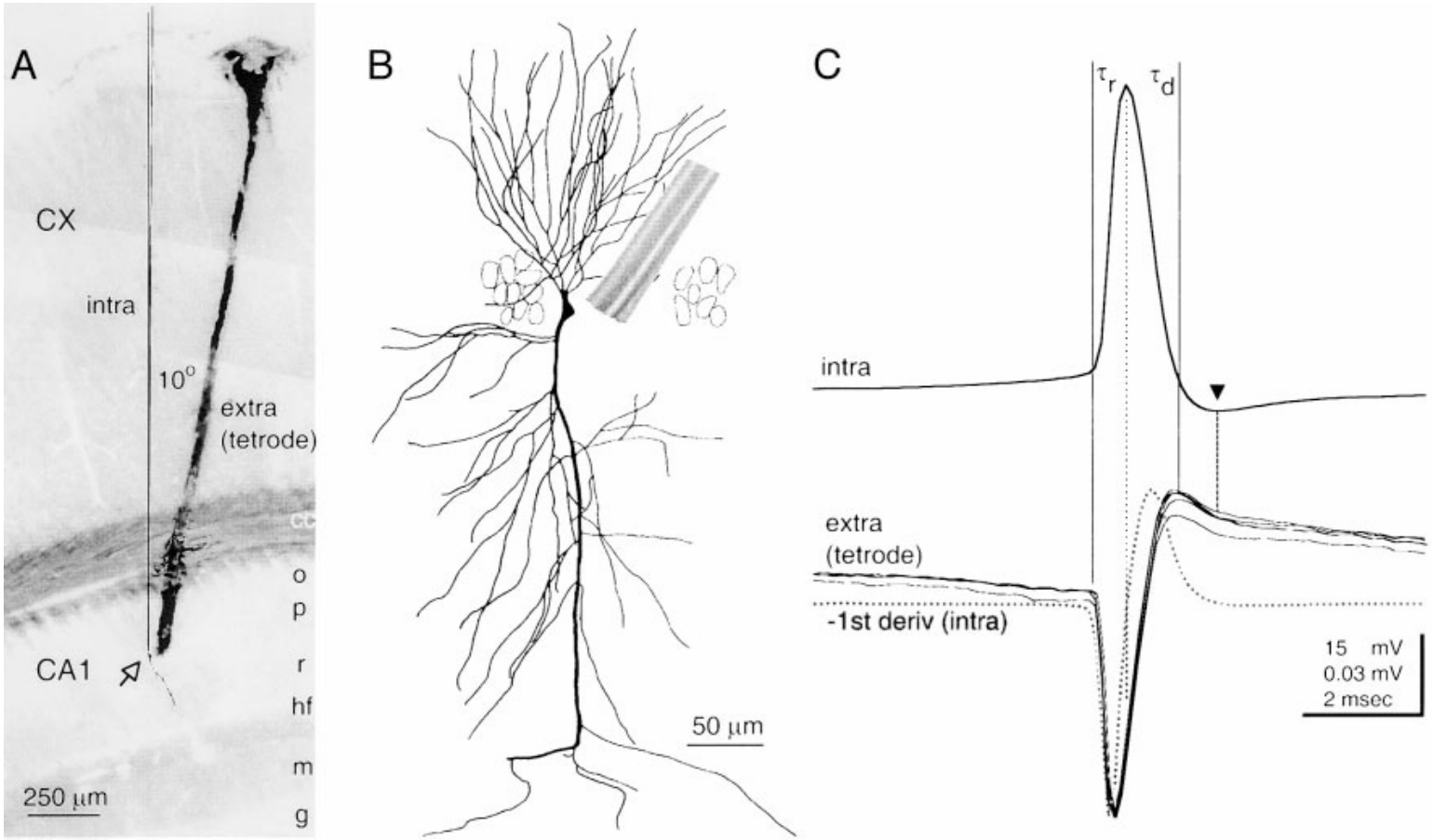




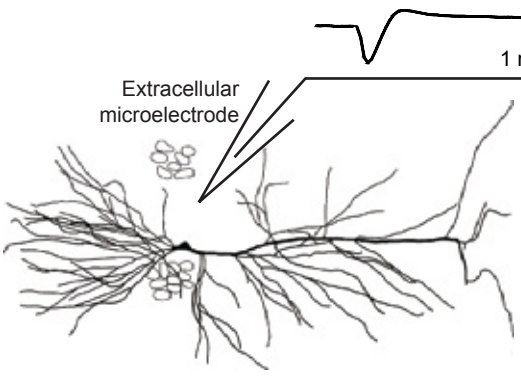


chapt_5_steady_state_dc.eps

Intra-extra Recording



Extracellular waveform is *almost* minus derivative of intracellular



Impedance conversion at the headstage

>> 1 GΩ input resistance
< 10 kΩ output resistance

Pre-amplification

100-times with 10 µV to
10 mV dynamic input range

Commutator

High-pass filtering

1 Hz for local field data or
~ 300 Hz for solely spike data

Post-amplifier

10- to 100-times with100 µV
to 1 V dynamic input range

Antialias filter

~ 10 kHz cut-off frequency

Sampling

Analog-to-digital conversion
at ~ 30 kHz and ± 14 bits

Raw field potential

Numerical filtering

600 Hz to 6 kHz band pass
with zero-phase

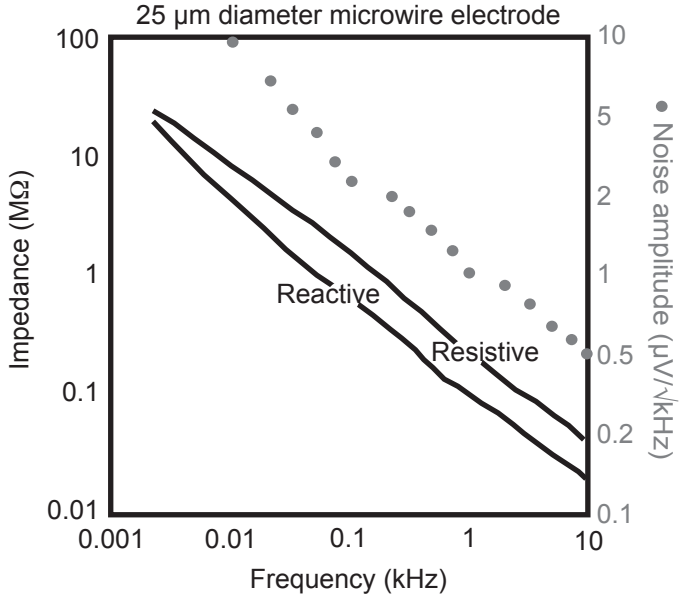
Detection of candidate waveforms

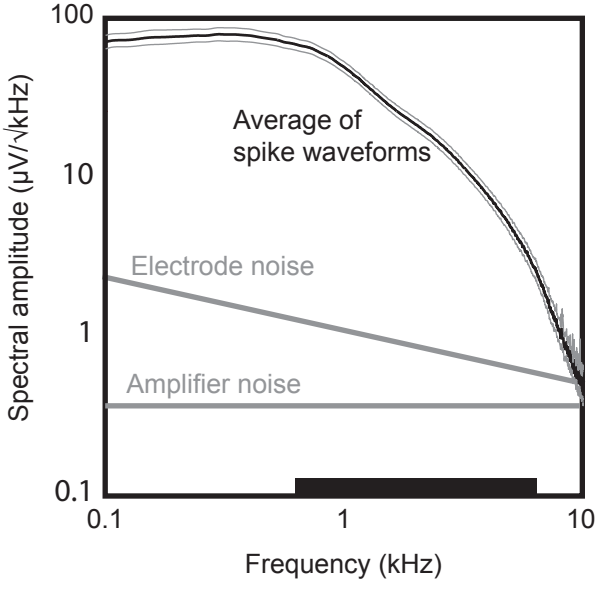
Alignment of waveforms

Segregation of waveforms with similar shape into groups

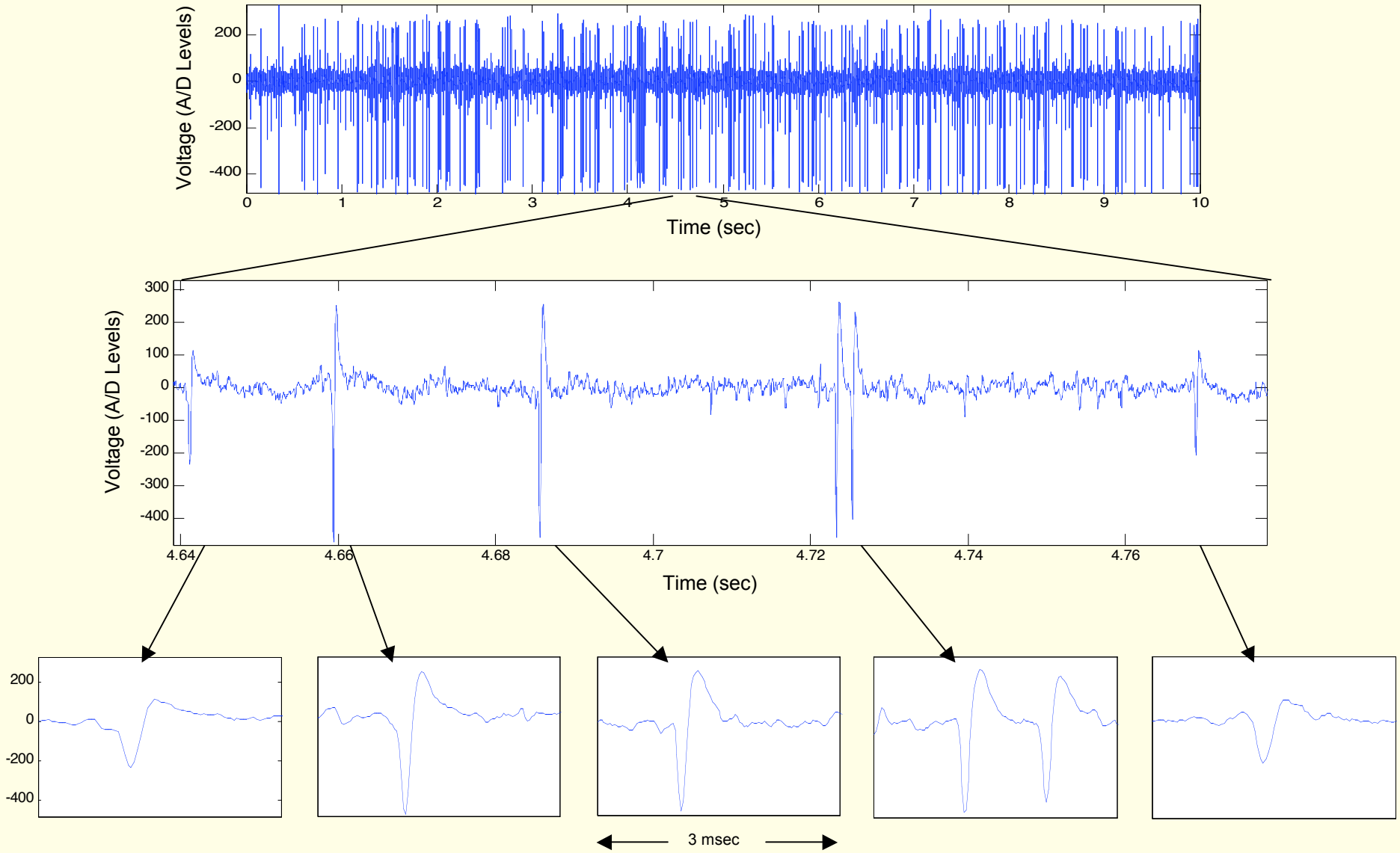
Quality assessment calculations

Time series of spike events



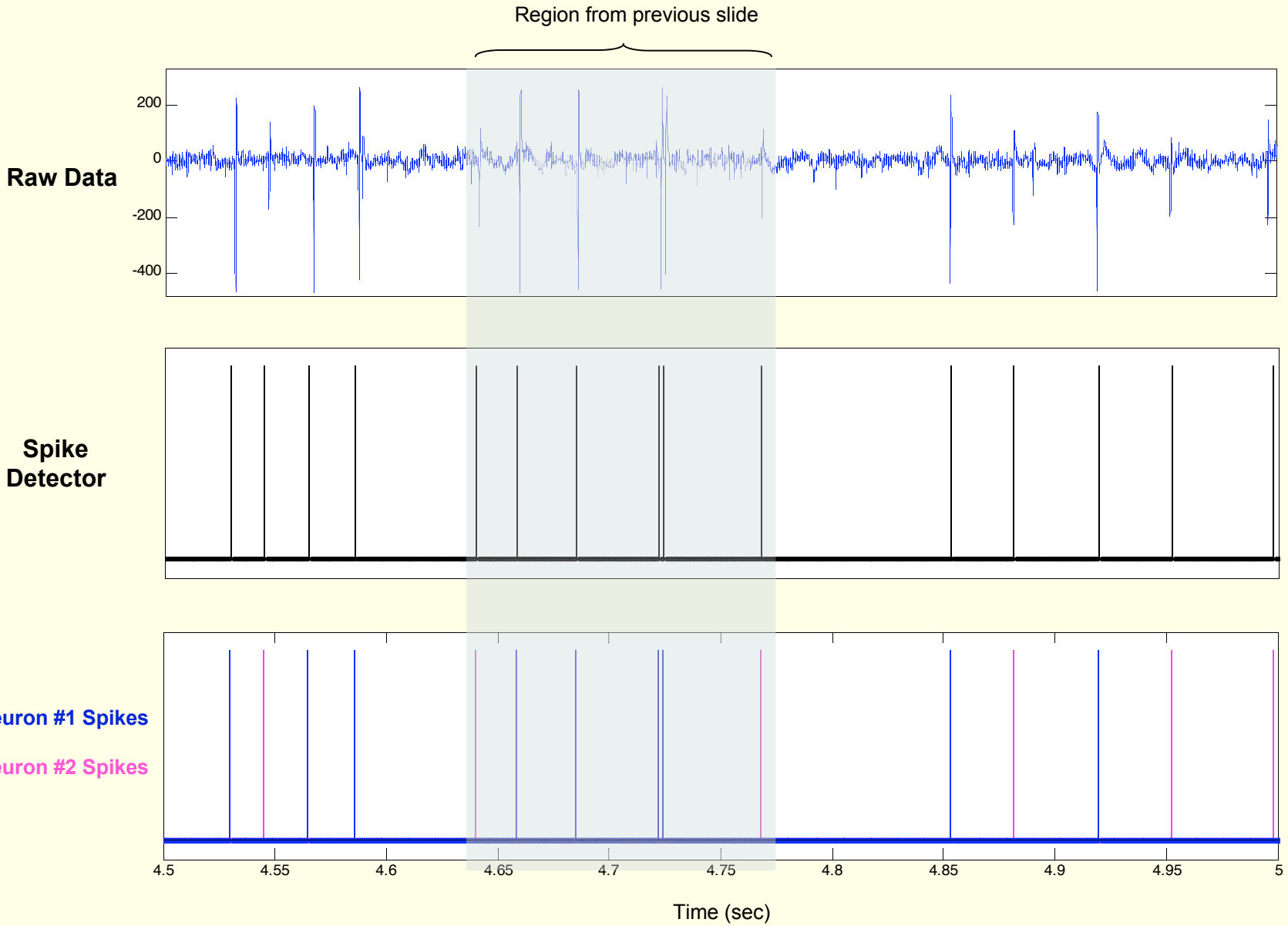


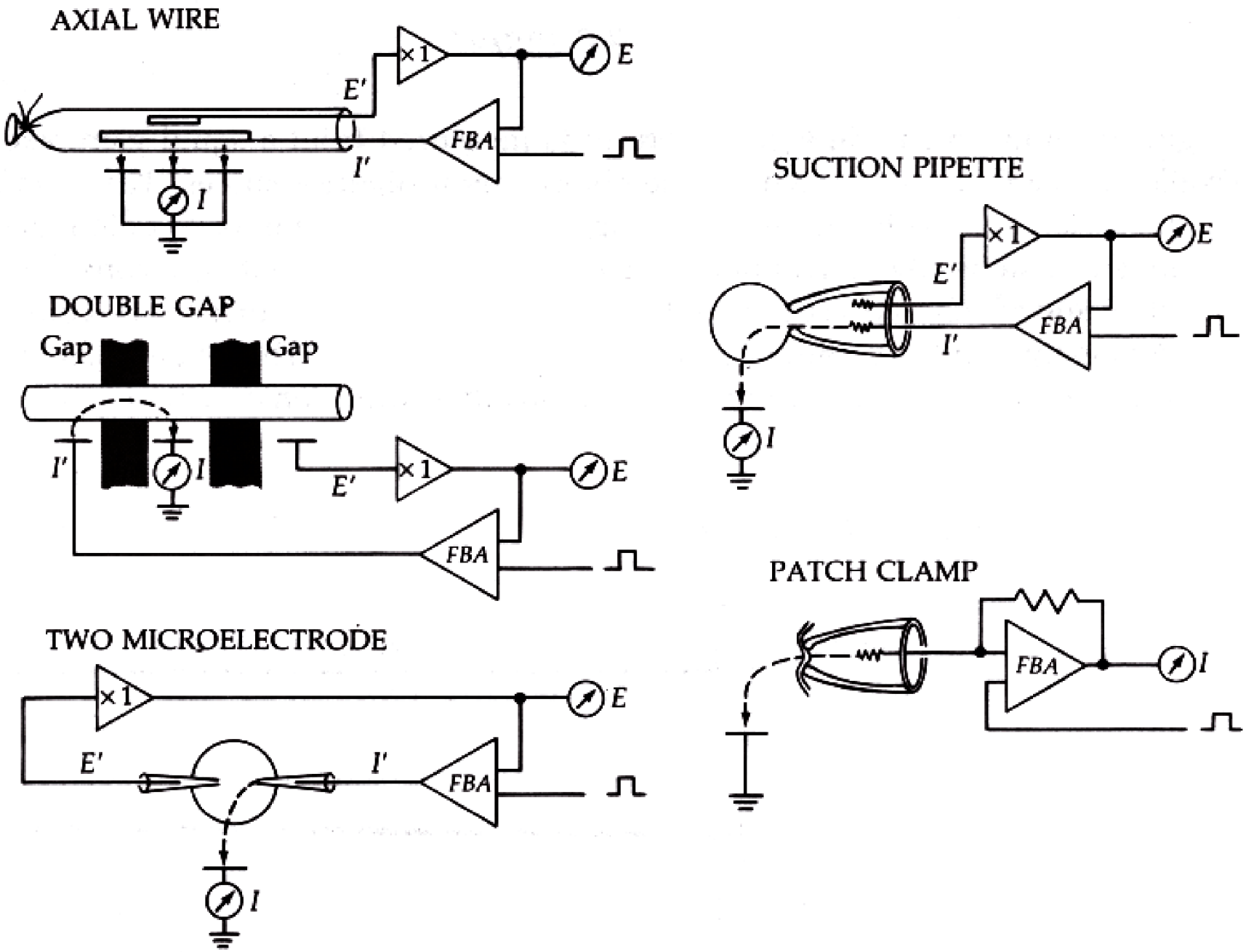
THE PROBLEM: Multiple Neural Signals



Primate retinal ganglion cells, courtesy of the lab of Dr. E.J. Chichilnisky

THE GOAL: Spike Times of Single Neurons

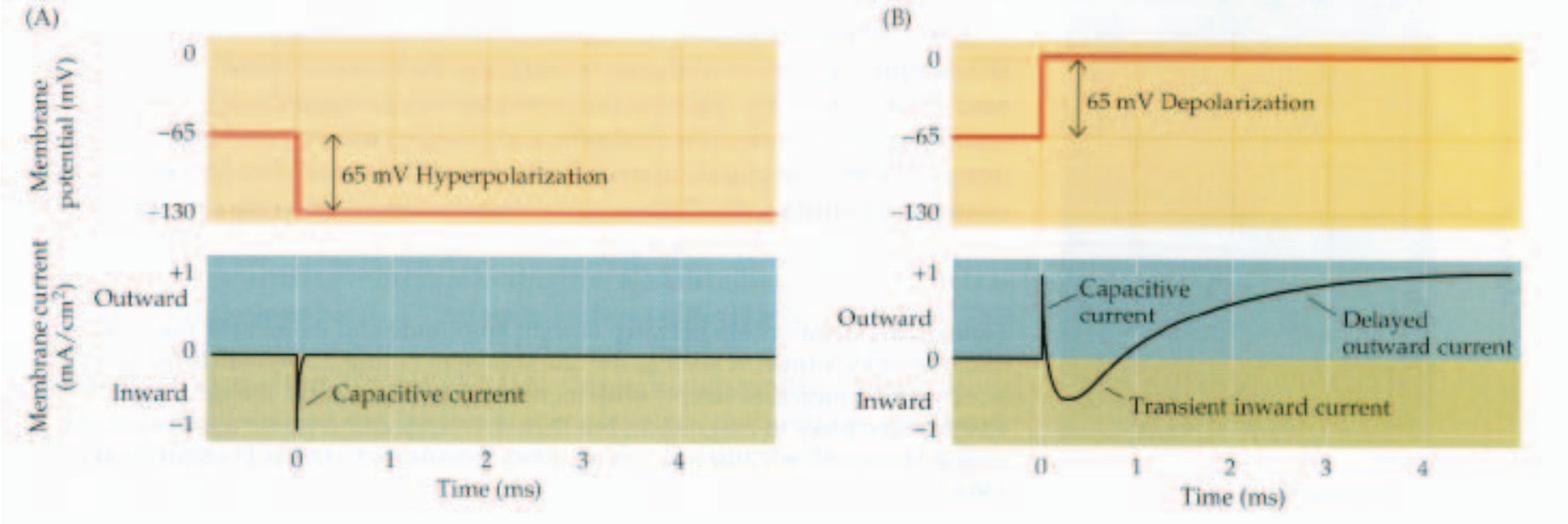


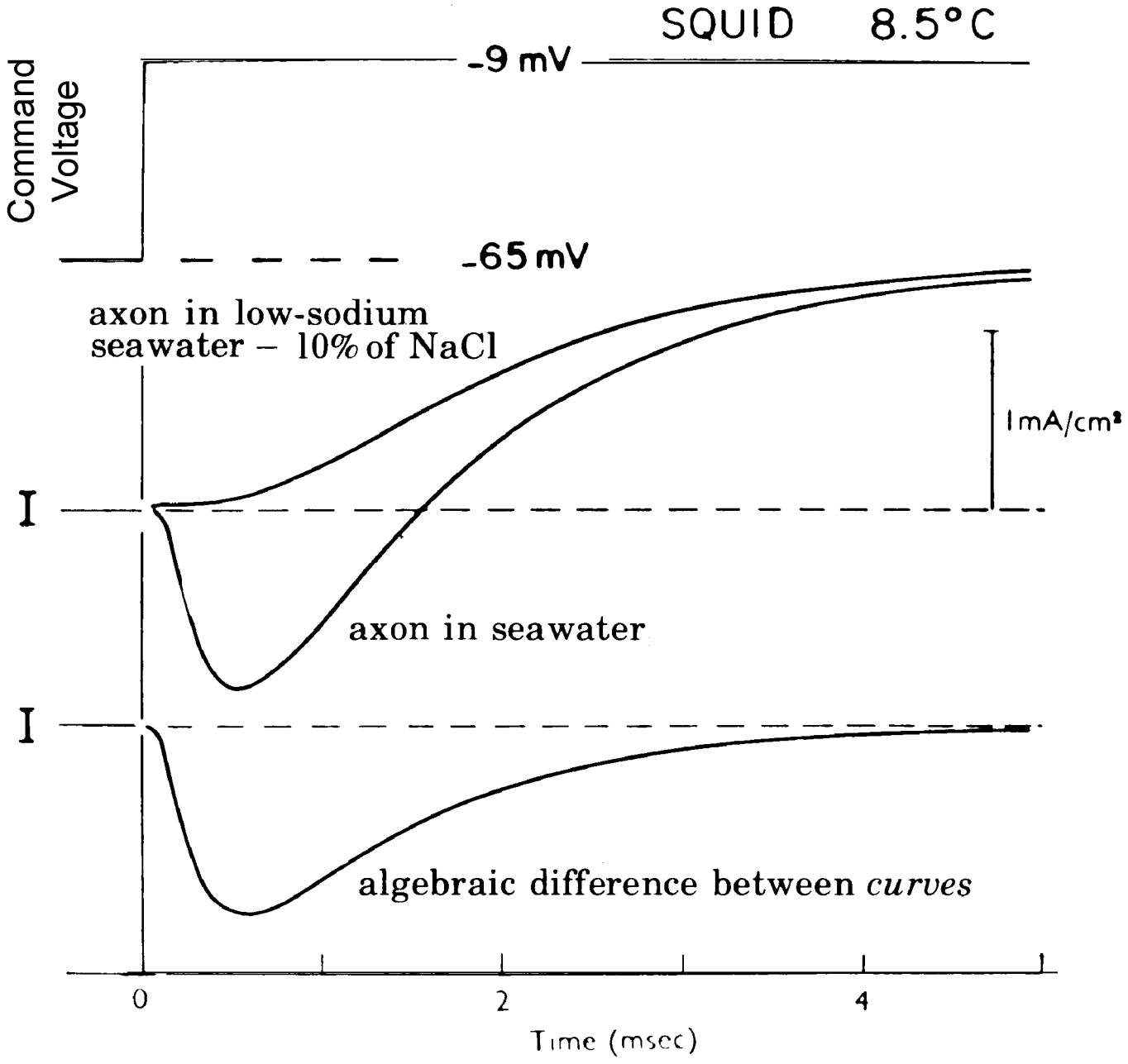


chapt_7_voltage_clamp.eps

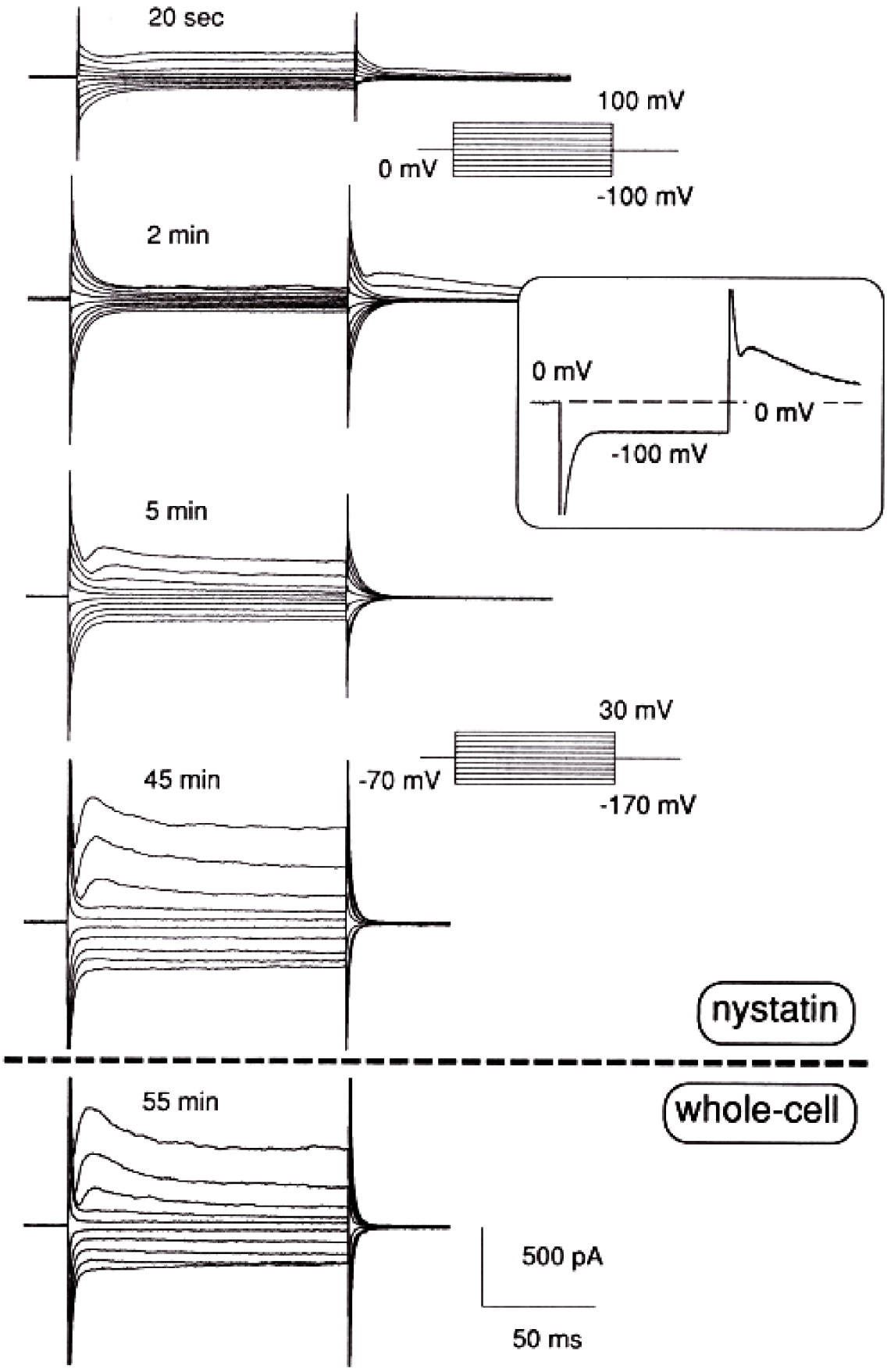
METHODS: Voltage Clamp II

- Capacitive current when applying voltage command
- Inward and outward currents when depolarizing

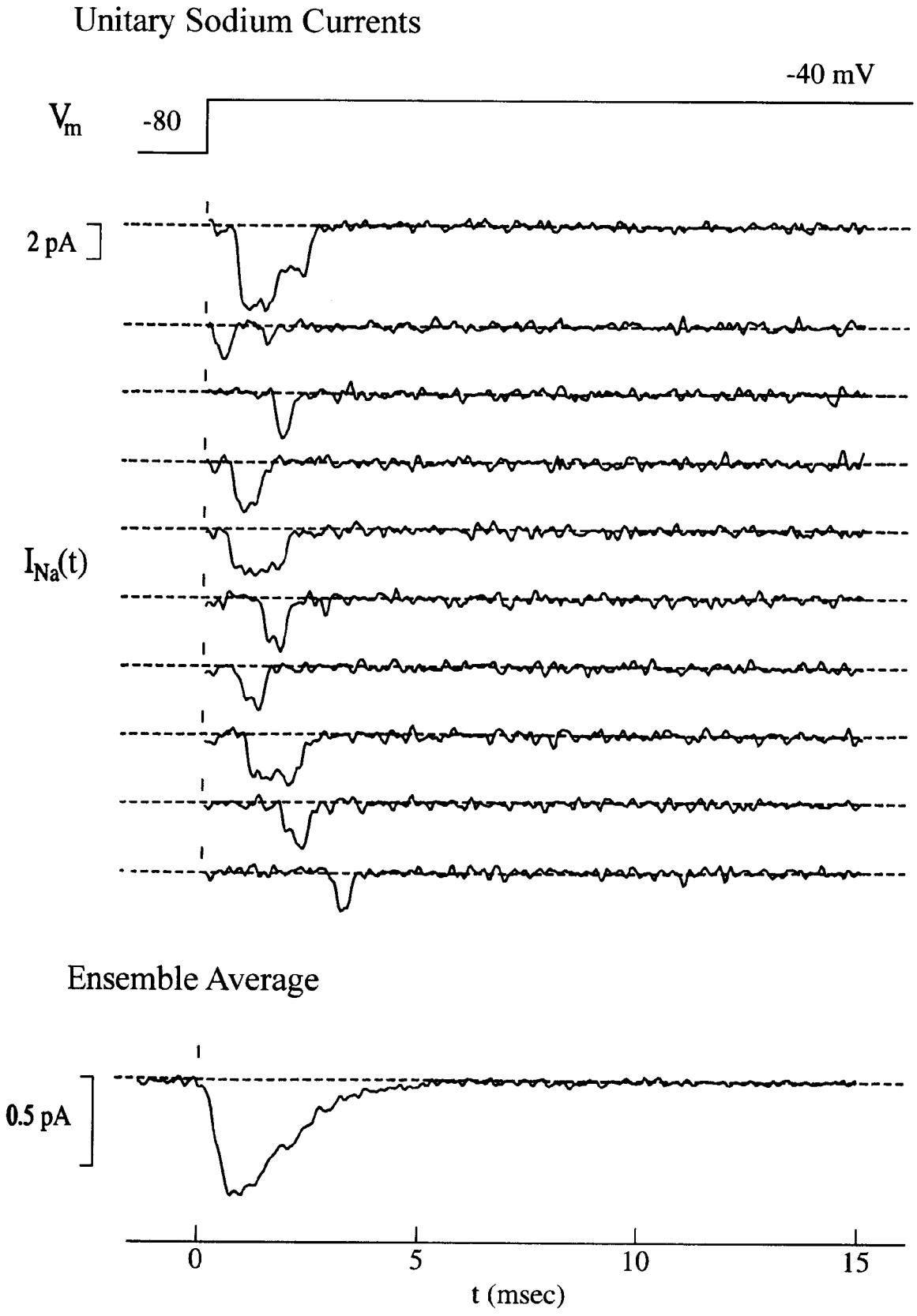




chapt_8_volt_clamp.eps



chapt_6_electrodes_2.eps



chapt_8_channel_sum.eps