

Fig. 1. Nuclei were isolated from thin slices of cerebellar cortex.

a and b, images of a sagittal slice of cerebellar cortex used for isolation of Purkinje and granule neuron nuclei.

c, a laser scanning microscope image of a nucleus isolated from a Purkinje neuron.

d, a fluorescent image of the same nucleus loaded with fluo-3.

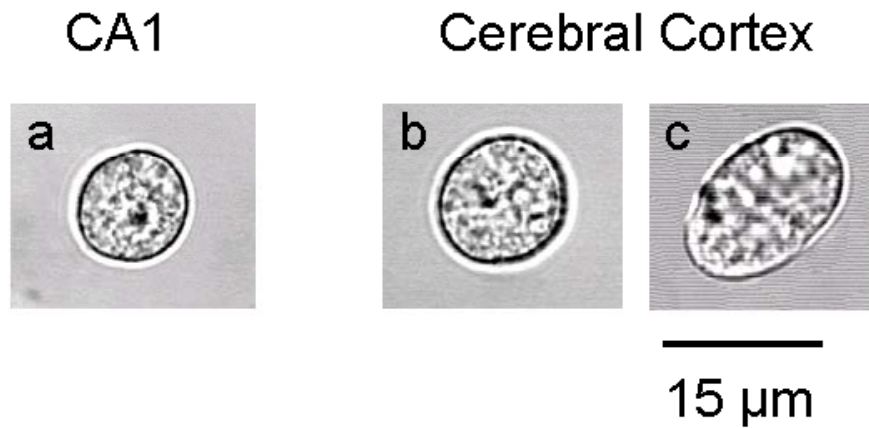


Fig. 2. Methods developed in this paper can be used for isolating nuclei from any type of central neurons.

a, a nucleus isolated from a hippocampal CA1 pyramidal neuron.

b and **c**, two nuclei isolated from pyramidal neurons of rat cerebral cortex.

All images were obtained from unstained live nuclei with a laser scanning microscope.

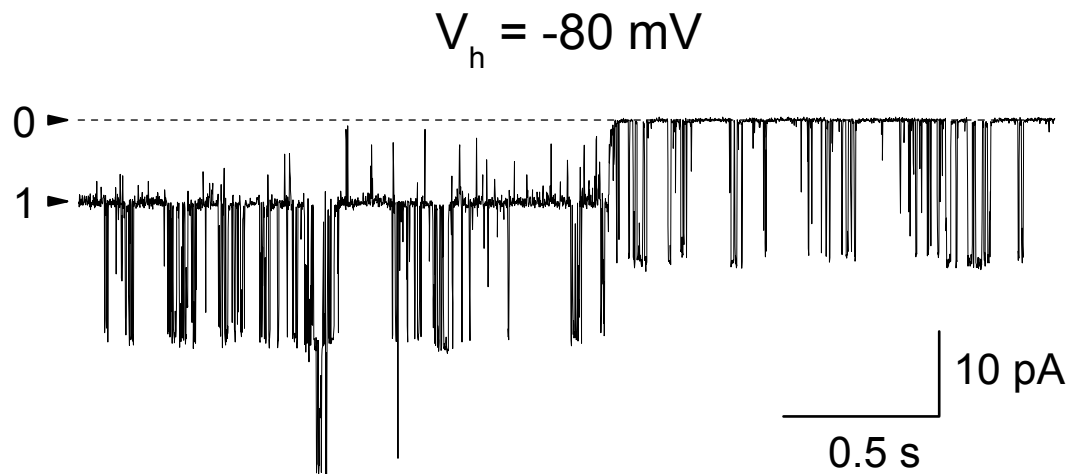


Fig. 3. Large-conductance cationic and InsP_3 -activated channels coexist in the inner nuclear membrane of Purkinje neurons.

The dotted line shows the zero current level. At the beginning of the trace the large-conductance cationic channel is open. The arrowheads indicate the levels of closed (0) and open (1) states of the channel. InsP_3 -activated channel activity is seen as short-lasting downward deflections from the zero-current level or is superimposed on the cationic channel current.

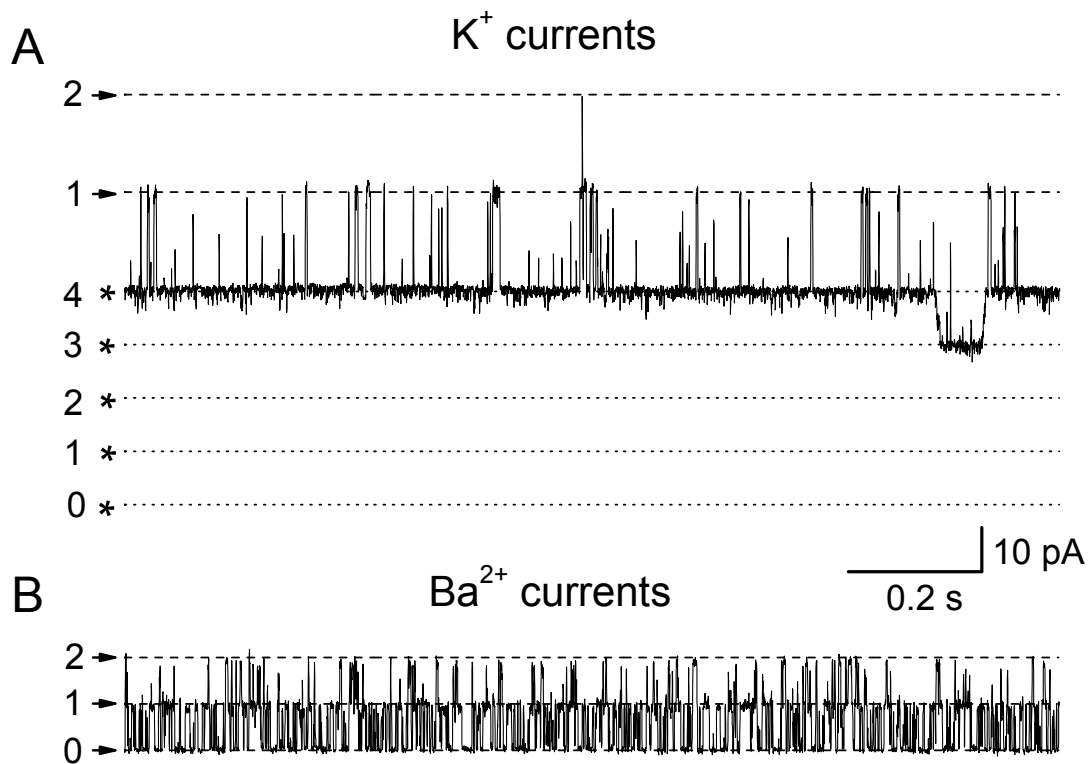


Fig. 4. Open probability of InsP₃-activated channels depended on ion species carrying current through the channel.

Two patches of the inner nuclear membrane both containing two InsP₃Rs.

When the current was carried by K⁺ (A) open probability of InsP₃-activated channels was much lower than it was carried by Ba²⁺ (B; P_o= 0.036 and 0.32 respectively). In A the patch contained four large-conductance cationic channels (their current levels shown by dotted lines and asterisks) superimposed on InsP₃R activity (indicated by dashed lines and arrows). In A and B the patch pipettes were filled with respectively KCl and BaCl₂ solutions, in both experiments bath solution contained KCl solution containing 2 μM InsP₃, 0.5 mM ATP and 250 nM free Ca²⁺. Holding potential was 60 mV.

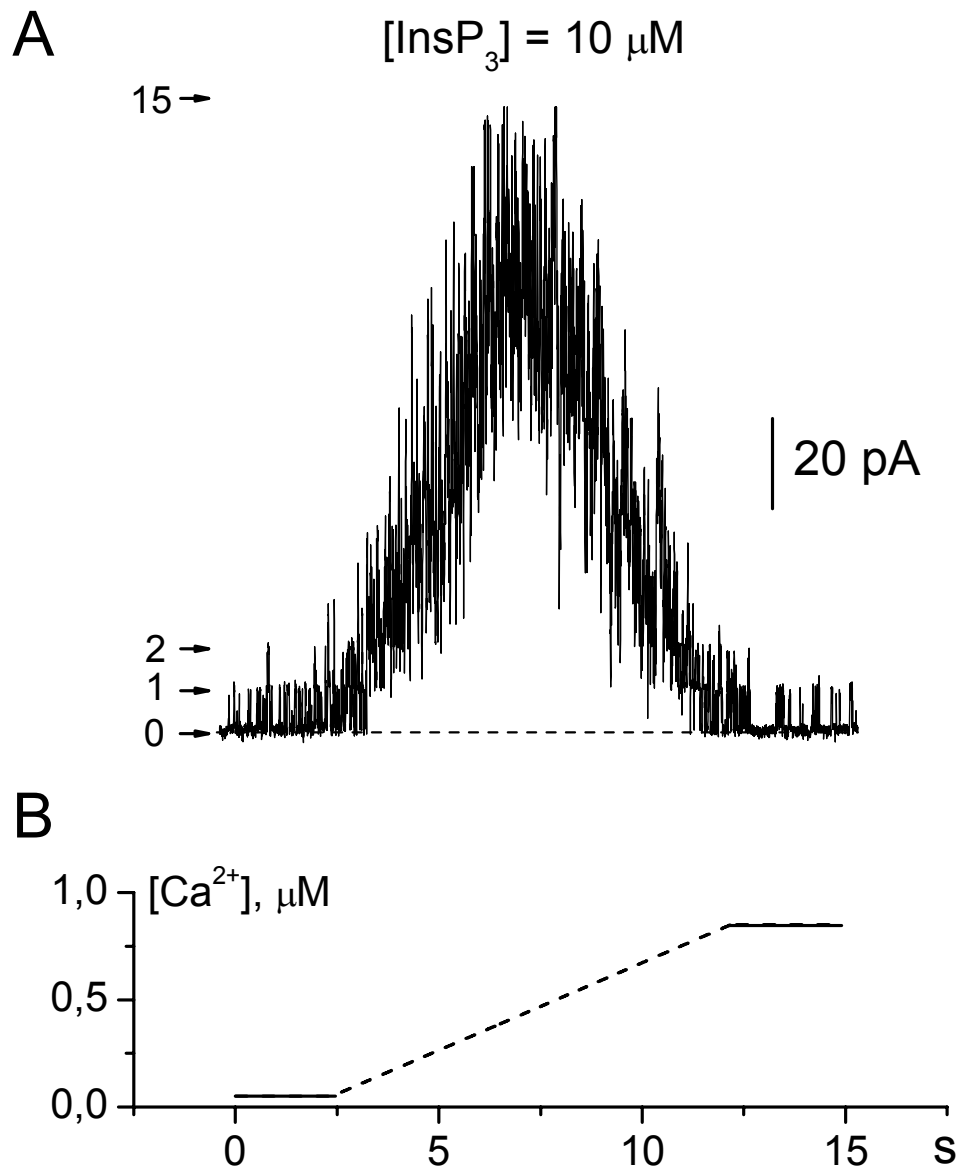


Fig. 5. Biphasic regulation of InsP_3Rs in the inner nuclear membrane of Purkinje neurons by Ca^{2+} .

A, at the beginning of the record bath was filled with KCl solution containing 50 nM free Ca^{2+} which was gradually substituted with solution with higher Ca^{2+} concentration (B) so that in the end of the record $[\text{Ca}^{2+}]_i$ was 850 nM. The number of simultaneously open InsP_3 -activated channels indicated by figures near arrows on the left. Concentrations of all other components remained unchanged throughout experiment – InsP_3 , 10 μM ; ATP, 0.5 mM. The rise in $[\text{Ca}^{2+}]_i$ was not strictly linear and is roughly shown in B by a dashed line.