### Title and Subtitle
Role of spatially distributed ion channels in single neuron computation

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Department of the Navy
Office of Naval Research

### SUPPLEMENTARY NOTES
none

### Abstract (Maximum 200 words)
Towards the overall goal of making an accurate mathematical model of a neocortical pyramidal neuron, (1) the electrical properties of Na\(^+\) and Ca\(^{2+}\) channel subtypes were measured in sufficient detail to construct a quantitative empirical model, and (2) antibodies were raised against the α\(_1\) subunits of these channel subtypes in order to determine their spatial distribution and relative density using quantitative immunocytochemistry.
INTRODUCTION

The overall purpose of this research was to elucidate the role of spatially distributed ion channels, specifically voltage-gated Na$^+$ and Ca$^{2+}$ channels, in the transformation of synaptic input to spike output in neocortical pyramidal neurons. The immediate goals during this grant period were to determine (1) the electrical properties of these ion channels and (2) their spatial distribution and relative density along the initial segment, soma and dendrites. Ultimately, these quantitative measurements can be combined to create a mathematical model of the pyramidal neuron. This model can be analyzed to determine the functional consequences of the spatially distributed channels on neuronal excitability. Such a model is needed because complex spatio-temporal interaction between ion channels at different locations preclude functional analysis by direct recording of electrical activity.

The determination of ion channel properties was done in the laboratory of Dr. Schwindt. These studies consisted of whole-cell and single-channel recordings of ionic currents associated with channel activity. These studies focused on the persistent Na$^+$ current, whose mechanism was previously unknown, and the current flowing through L-, N- and P-type Ca$^{2+}$ channels. The goal of these studies was to obtain sufficient quantitative data on the electrical properties of these channels to construct a quantitative empirical model suitable for use in the computer model of the whole-neuron referred to above.

The determination of ion channel location was done in the laboratory of Dr. Catterall. These studies consisted of the development of (1) specific antibodies directed against the $\alpha_1$ subunits of specific Na$^+$ and Ca$^{2+}$ channels subtypes and (2) a labeling procedure to visualize the antibodies the cell membrane and allow quantitative measurements of location and relative density of each channel subtype. The goal of these studies was to provide the spatial information needed for the whole-neuron model referred to above.

The results achieved during this grant period are summarized below in two sections, the first section describes the electrophysiological results, and the second describes the localization results. In this summary, I refer to published papers supported by this grant. These papers are numbered and listed alphabetically beginning on page 3. Reprints of those papers which have already been published are included with this report.

RESULTS

Electrophysiological Studies

Our original hypothesis was that the persistent sodium current, $I_{NaP}$, arose from a unique type of Na$^+$ channel, distinct from the one responsible for the transient Na$^+$ current and corresponding perhaps to the RI Na$^+$ channel subtype which is confined to the neuronal soma. As described in References 1-3, we found only one type of Na$^+$ channel on the soma of neocortical pyramidal neurons. This Na$^+$ channel displayed the usual transient gating mode, but two additional gating modes were apparent during long-
lasting depolarizations. Computations based on the single-channel data predicted that the late gating modes should give rise to a persistent whole-cell $\text{Na}^+$ current (3), and this was confirmed subsequently by whole-cell recordings from the same type of neurons (4,5). The two late gating modes appear to give rise to two kinetically-distinct components of $I_{\text{NaP}}$ (5). It is unlikely that only the RI channel causes $I_{\text{NaP}}$. The late gating modes and the whole-cell $I_{\text{NaP}}$ are seen both at early ages where only the RIII Na$^+$ channel type is present and also at later times when the RI channel type should also be present. Other researchers working on Na$^+$ channels from muscle and even cloned Na$^+$ channels have observed similar modal gating. Thus, it appears that any Na$^+$ channel subtype can give rise to an $I_{\text{NaP}}$, and we need no longer distinguish between Na$^+$ channel subtypes (e.g., RI, RII, etc.) in our single-neuron labeling studies. This will greatly expedite and simplify the gathering of the required data. Whole-cell recording has resulted in a description of the conductance underlying $I_{\text{NaP}}$ that will be useful for modeling purposes (5).

**Calcium Channels**

The goal of this research was to construct an empirical model of the high-threshold voltage activated Ca$^{2+}$ current (HVA current) of neocortical pyramidal neurons. These studies also have been completed (6,7). The HVA current is composed of pharmacologically-separable components, which probably correspond to activation of L, N and P channel types. Thus, an important question was whether these components differ in kinetics and voltage dependence. We have found that the HVA current of neocortical neurons may be treated as homogeneous with respect to these properties. That is, all components have the same voltage dependence and kinetics, irrespective of their sensitivity to pharmacological agents. This finding will greatly simplify the analysis of the role of these channels in neuronal computation once quantitative measures of channel density are available. Based on our measurements, we constructed a Hodgkin-Huxley-like model of the HVA current, and we found that this model could satisfactorily predict the HVA current evoked both by voltage clamp and a train of action potentials in dissociated neocortical neurons (7).

**Ca$^{2+}$-activated Channels**

Two other studies of the effects of Ca$^{2+}$ influx and accumulation in neocortical pyramidal cells were performed during this period. In one of these studies (13), two distinct types of Ca$^{2+}$-activated K$^+$ currents in neocortical neurons were described in detail, and it was concluded that the dependence of one of these currents on Ca$^{2+}$ was indirect, possibly through a Ca$^{2+}$- dependent enzyme intermediary. The other paper (12) showed that the response properties of the neurons is highly dependent on intracellular Ca$^{2+}$ buffering in an unexpected way. In addition, two voltage-gated currents (including $I_{\text{NaP}}$) appear to be modulated indirectly by intracellular Ca$^{2+}$ levels.

**Other Work**

At the invitation of our ONR Scientific Officer, Dr. Thomas McKenna, a book chapter was written describing the control of input-output properties of neocortical neurons by intrinsic membrane conductances (11).

**Localization of Ion Channels**

**Calcium Channels**

At the start of this project our sole available Ca$^{2+}$ channel antibody was directed against the $\alpha_2$ subunit of the L type Ca$^{2+}$ channel in brain. During this grant period extensive work has been done on the identification and characterization of the various subtypes of Ca$^{2+}$ channels. The work which resulted in publication thus far includes the determination of the primary structure of the rbB-I Ca$^{2+}$ channel $\alpha_1$ subunit (also known as class B Ca$^{2+}$ channels). A polyclonal antiserum against this protein selectively
immunoprecipitates $^{125}$I-labeled $\omega$-conotoxin-binding sites from rat forebrain. Expression of the rbB-I channel is restricted to the nervous system and cell lines that express the N-type Ca$^{2+}$ channel (8). In a related study, a site directed anti-peptide antibody, CBNI, that recognizes the $\alpha_1$ subunit of rat brain class B Ca$^{2+}$ channels (rbB-I) immunoprecipitated 43% of the N-type Ca$^{2+}$ channels labeled by $^{125}$I-$\omega$-conotoxin. In addition, the CBNI antibody recognized proteins of 240 and 210 kD, suggesting the existence of two forms of this $\alpha_1$ subunit. Immunocytochemical studies demonstrated that N-type channels recognized by CBNI were localized predominantly in dendrites; both dendritic shafts and punctate synaptic structures were labeled. The cell bodies of some pyramidal cells in layers II, III, and V of dorsal cortex, Purkinje cells, and scattered cell bodies elsewhere in the brain were also labeled at low levels (14, 15).

In another study we have identified and localized the protein product of the two different class C and class D L-type Ca$^{2+}$ channel $\alpha_1$ subunits. Two antipeptide antibodies specific for the class C or class D $\alpha_1$ subunits, known as CNC1 or CND1 respectively, were produced. Fully 75% of the neuronal L-type channels labeled by 3H-PN200-110 could be immunoprecipitated by CNC1, and 20% was immunoprecipitated by CND1. Immunoblotting revealed the existence of two sizes of the Class C L-type $\alpha_1$ subunit and two sizes of the class D L-type $\alpha_1$ subunit. Immunocytochemical studies using CNC1 and CND1 antibodies revealed that the $\alpha_1$ subunit of both L-type Ca$^{2+}$ channels is localized mainly on neuronal somata and proximal dendrites (10). Staining in distal dendrites was usually very faint but detectable. The relatively high concentration of L-type Ca$^{2+}$ channels in cell bodies and proximal dendrites is in contrast to the predominant localization of N-type Ca$^{2+}$ channels in distal dendrites.

Because of these exciting new developments we are able to greatly expand the scope of our investigation. Up to now we could only label the $\alpha_2$ subunit of L channels. Not only can we more accurately localize subtypes of these L channels (by using $\alpha_1$-directed antibodies), but we now will be able to visualize the spatial location of the three Ca$^{2+}$ channel subtypes (L, N and P) that are known to underlie the HVA Ca$^{2+}$ current in neurons (9).

**Sodium Channels**

Measurement of the spatial localization of Na$^+$ channels in single neurons has proceeded using confocal microscopy. In light of our physiological results (see above) suggesting that any type of Na$^+$ channel can give rise to a persistent Na$^+$ current, we can now utilize an antibody (AbSP20) that recognizes all types of Na$^+$ channels. This will expedite and simplify the gathering of the required data.

**Published Material Resulting From ONR Funding:**


